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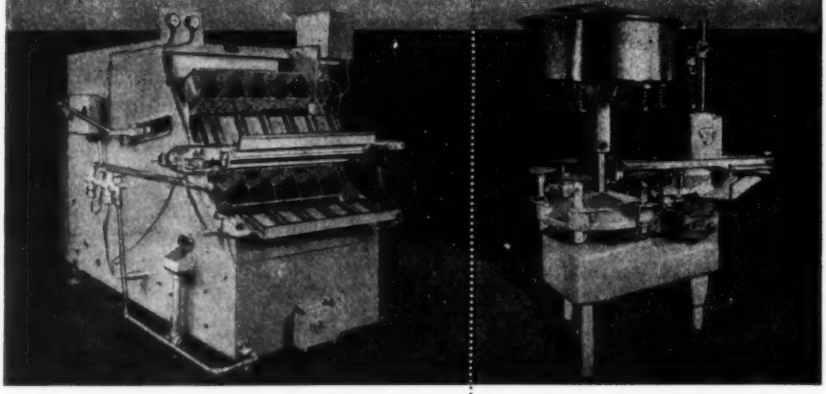

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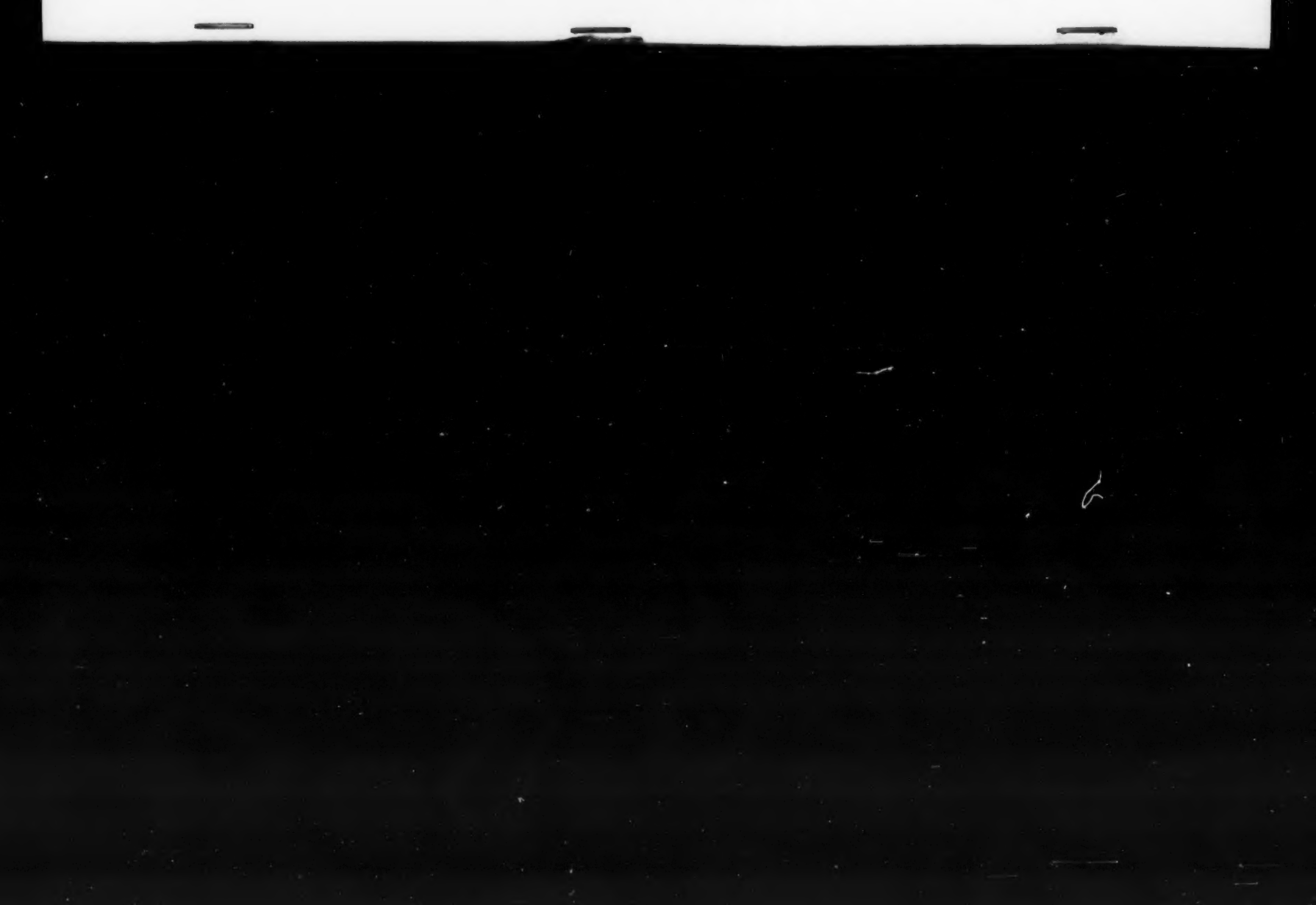

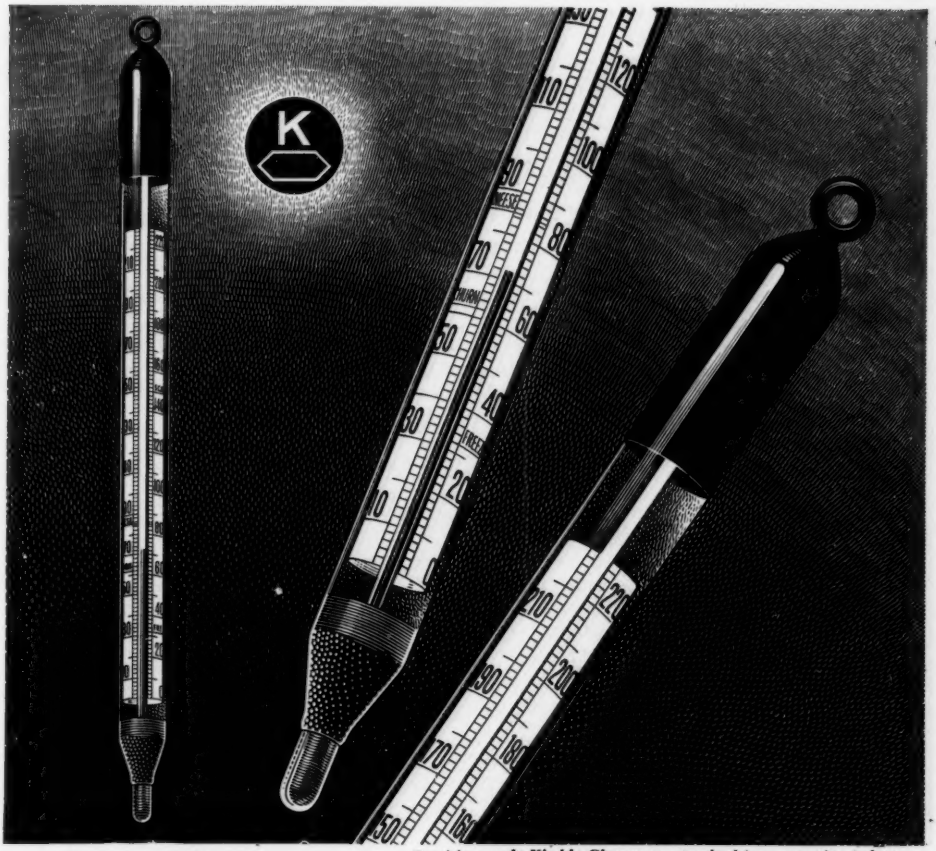

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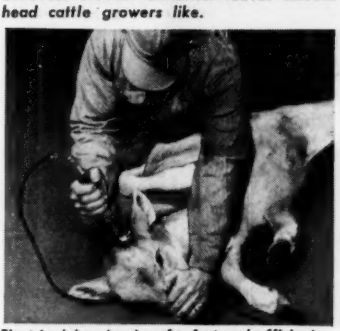

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXVII November, 1954 NUMBER 11

THE EFFECTS OF HOURLY MILKING WITH THE AID OF INTRAVENOUS INJECTIONS OF OXYTOCIN[1]

J. D. DONKER,[2] J. H. KOSHI, AND W. E. PETERSEN

Dairy Husbandry Department, University of Minnesota

There has been a renewed interest in the physiological significance of oxytocin since Ely and Petersen *(5)* presented their report on the modern concept of the complex milk ejection reflex. Prior to that work there were reports on the use of posterior pituitary extract which stated that there was a transitory rise in the amount of milk obtained following injection *(6, 8, 12)*. Recent work has shown that milk production may be increased when oxytocin is administered at each milking over extended periods of consecutive regular milkings *(2, 3, 15)*. Shaw *(13)* and Smith *(14)*, reporting on the effects of oxytocin administered at 2-hour intervals, were not in complete agreement as to the effect of the hormone. Shaw observed that there was residual milk in the second milk sample collected when milking was carried out at 2-hour intervals. Both observed a decrease in butterfat percentage of milk samples following oxytocin injection if the first few milkings which contained residual milk were disregarded. Smith found a definite increase in production, whereas it was Shaw's opinion that production was not significantly changed.

Johansson *(9)* has attributed the main cause of daily fat percentage variability under normal conditions to the variations in completeness of milk evacuation. He reported greatly reduced milking-to-milking variation in the fat per cent of milk obtained at regular intervals when oxytocin had been administered. This observation was explained as being due to a more nearly complete removal of the last fat-rich milk.

Brown *et al. (1)* demonstrated that milk samples drawn hourly, even though there was not complete evacuation, differed greatly in lactose per cent from hour to hour. Since it has been reasonably well established that the repeated intravenous administration of sufficient quantities of oxytocin will empty the gland of preformed milk, its employment for milking at short intervals would contribute to a knowledge of the variations in quantity and quality of milk produced and of the effect of accumulating milk in the alveoli upon the character and quantity of secretion.

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[1] Paper No. 3057, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Present address: Dairy Department, University of Georgia, Athens.

With these objectives in mind, an experiment was set up in which hourly milking with the aid of oxytocin administered intravenously was carried out over various periods. After primary observations, the intervals between milkings were increased to note the effect on milk secretion.

EXPERIMENTAL METHODS

Four cows were used in five trials on three different occasions in which a period of twice daily milking was followed by a series of hourly milkings. In the first trial, hourly milking continued for 60 hours, in the next three for 76 hours, and in the last, 156 hours. In the first four trials, hourly intervals between milkings were followed by a series of increasing time intervals between milkings. There were two consecutive milkings following a given time interval, and then the interval was increased. In the first trial, the intervals were first increased from 1 to 2 hours and then by 2-hour increases to 18 hours. In the next group of three trials, the increase was from 1 to 4 hours and by 4-hour steps up to 16 hours. Each of these milkings was carried out by injecting oxytocin prior to hand milking. Oxytocin was administered intravenously in the amount of 20 I.U. per milking. Normal milking procedures were resumed after the last long or hourly milking interval.

The milk from each milking was weighed and analyzed for butterfat content by the Babcock method. In the last four trials the saponification numbers of the butterfats of individual samples were determined by conventional methods. Correlations were run between saponification numbers of fat samples and the observed butterfat percentages of the milk from which the fat was removed. In the fifth trial, approximately every fifth hourly sample was analyzed for lactose content and the nitrogen partition. The nitrogen partition was determined by the method outlined by Larson and Jenness *(11)*. Lactose determinations were made colorimetrically by a method devised by Gander *(7)* in which lactose is freed of the milk fat and proteins and color developed by a phenol-sulfuric acid reagent.

The 156-hour trial was divided into 15 ten-hour periods for statistical analysis to determine variability within periods to show general trends. The first 4 and the last 2 hours were not considered; the first period because of amounts of normal residual milk being present and the latter period as a matter of convenience.

Observations within the experimental period were compared to a 1-week pre-experimental period and a post-experimental period of 10 days. A 3-day adjustment period was allowed after the last oxytocin injection prior to the post-experimental observations.

Milk obtained with the aid of oxytocin which was in excess of that normally obtained at a milking is designated "complementary milk" in accordance with the terminology previously used *(4)*.

RESULTS

The production of total milk and butterfat, expressed as a per cent of the pre-experimental average rate is shown in Figure 1, by 4-hour periods. It is

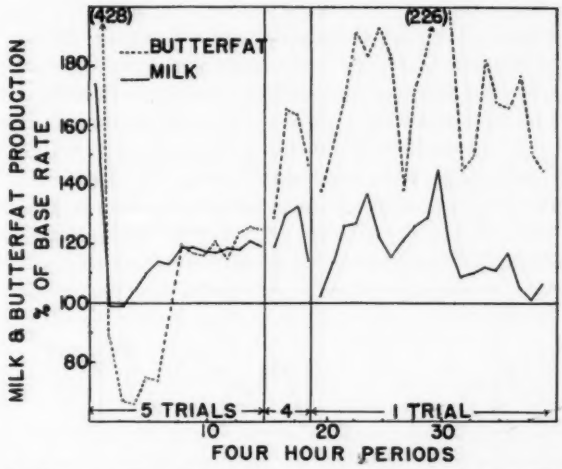

FIG. 1. Production of milk and butterfat in the hourly oxytocin milking period. The values are from the average of four consecutive milkings expressed as a percentage of the pre-experimental base rate. Five trials carried through 60 hours, four through 76 hours, and one through 156 hours.

seen that the yields were high in the first 4 hours. The average hourly production of total milk and butterfat for this period was 174% and 428%, respectively, of the base rate. In the next two periods the average rate of milk secretion was approximately normal, whereas the fat was depressed to 67% of the base rate. Between the fifth and tenth periods (20 to 40 hours) milk secretion averaged 116% of the base rate and the fat, 100%. The hourly fat production remained below normal for a whole day and then closely paralleled total milk production from the seventh to the 15th periods. In this latter interval, therefore, the

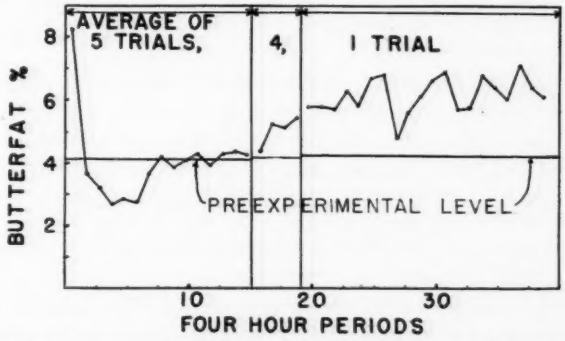

FIG. 2. Butterfat percentages of milks from hourly milking periods in which milk was ejected by oxytocin. Five trials carried through 60 hours, four through 76 hours, and one through 156 hours. Values are averages of four consecutive hourly milkings for the group or individual.

butterfat content of the milk was approximately normal. From the 15th period (60 hours) onward, the average fat production in 4-hour periods ranged from 129 to 225% of the base rate. The mean value for periods beyond 60 hours was 168%. Generally, the milk and butterfat curves were parallel, but the rate of increase for milk secretion was not as great as that for butterfat. The mean 4-hour period value beyond 60 hours for milk production was 119%, and thus the butterfat percentages were significantly above the base value. The hourly average butterfat per cent of hourly milk samples by 4-hour periods is presented in Figure 2. The increase in variation of the butterfat per cent from period to period after 60 hours is due to the smaller number of samples involved.

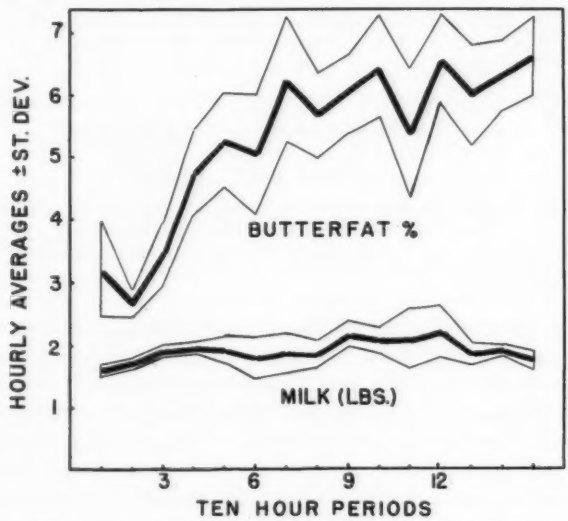

FIG. 3. Mean hourly milk production and butterfat percentages of milk in 10-hour periods for the hourly milking trial which was continued for 156 hours. A range of one plus and minus standard deviation surrounds the line of mean values. The first four and last two hourly milkings are excluded from the figure.

Figure 3 was constructed from data of the 156-hour trial. The mean values of hourly milk production and butterfat percentages together with a plus and minus standard deviation are presented for 15 consecutive 10-hour periods. The variability of the butterfat percentages of milk samples is greater on the average than that of the milk yields. The means of the relative deviations

$$\frac{(\text{standard deviation} \times 100)}{\text{mean}}$$

for hourly milk weights and butterfat per cents for the periods are 10.9 and 13.9%, respectively. In this trial, the pre-experimental milk secretion rate was 1.69 lb. per hour with 4.2% butterfat. The averages of the means for the 15 ten-hour periods for milk yields and fat percentages with the averages of the standard deviations for these values are 1.92 ± 0.21 lb. per hour and $5.30 \pm$

0.72%, respectively. The range between period means for milk secretion rate was from 1.60 to 2.22 lb. per hour and the range for fat per cent was from 2.66 to 6.59. Expressing the increased production attained as daily production, the data show that during the fifth day the cow on the 156-hour trial produced 51 lb. of milk containing 3.1 lb. of butterfat, compared to a base production of 41 lb. of milk and 1.7 lb. of fat daily.

The trend shown by the butterfat percentages was erratic, but there was first a definite drop followed by a marked upward trend from the 30th hour, tending to plateau slowly after the 70th hour.

The saponification numbers of the butterfat samples varied from 223 to 247 with a great deal of fluctuation from hour to hour without any detectable trend. The correlations between the butterfat percentages and the saponification numbers for the four animals used were as follows: 0.12, 0.16, 0.30, and 0.64.

In Table 1, total protein, casein, serum protein, and lactose values calculated on fat free basis for the pre-experimental and experimental milk samples are shown. Total protein, casein, and serum protein in the experimental samples fluctuated considerably from one hour to the next. There was no definite detectable trend. Protein contents were lower in the experimental than in the pre-experimental samples.

Lactose values also showed considerable fluctuation from hour to hour within the range shown in Table 1, without detectable trend and with no difference between the experimental and the pre-experimental milks.

TABLE 1

Per cent of total protein, casein, serum protein, and lactose in milk samples expressed on a fat free basis for the pre-experimental and experimental period

	Pre-experimental*	Experimental		
		Low	Mean	High
Total protein	4.30	2.48	3.41	3.79
Casein	3.67	2.03	2.90	3.29
Serum protein	0.63	0.32	0.51	0.62
Lactose	5.05	3.93	5.08	6.05

* Single sample obtained at the last pre-experimental milking.

The influence of increasing the interval between milkings following periods of hourly milkings is shown by the data presented in Table 2. Although butterfat production and total milk production both were above 120% of the base rate between 51 and 60 hours, the average milk yield per hour for the total hourly milking period beyond the first 4-hour period was relatively higher than the fat yield because secretion had been suppressed in the early part of the hourly milking period (124% for milk yield, 112% for butterfat yield.)

When the intervals between milkings were increased from 1 to 16 hours, the fat secretion rates for milkings for the intervals of 4, 8, 12, and 16 were 137, 142, 138, and 131% of the base rate, respectively. In each period, the secretion rate was above the highest rate recorded in the hourly milking period (127% between 51 and 60 hours). The milk secretion rates which followed the 4, 8, 12, and 16-hour intervals were 118, 123, 120, and 106% of the base rate, respectively.

TABLE 2

Production of milk and butterfat in the experimental period compared to control periods (average of four cows expressed as pounds per hour per cow and and as the per cent of the base period rate)

7 day base period		Experimental milking period (Hours between milkings)										10-day post exptl. period	
		1[a]		4		8		12		16			
(lb.)	*(%)*	*(lb.)*	*(%)*	*(lb.)*	*(%)*	*(lb.)*	*(%)*	*(lb.)*	*(%)*	*(lb.)*	*(%)*	*(lb.)*	*(%)*
						milk							
1.27	100	1.57	124	1.50	118	1.56	123	1.53	120	1.34	106	1.27	100
						butterfat							
0.052	100	0.058	112	0.071	137	0.074	142	0.072	138	0.068	131	0.052	100

[a] Three cows milked 76 consecutive hours, 1 cow 61 hours; first 4 hours of each excluded because of their content of complementary milk.

The secretion of milk per hour apparently did not materially change from the hourly rate of 124% until the intervals between milkings increased from 12 to 16 hours. The rate of milk formation dropped from 120 to 106% of the base rate with this 4-hour increase between milkings. The rate of milk and fat secretion returned to the pre-experimental values in the post-experimental period of normal milking procedures.

DISCUSSION

The first observation of significance was the fact that the average butterfat per cent and butterfat and milk yields were above the pre-experimental levels, considering the entire experimental period. Milk yields in the first 4 hours approached the pre-experimental levels some time before fat percentages, indicating that the amount of residual fat was of a greater magnitude and was more difficult to remove than the more fluid component of the residual milk. These observations tend to support the contention of other workers *(10, 13, 14)* that more than one oxytocin-milking is necessary to remove complementary milk. Johansson *(10)* found it necessary to inject oxytocin three times at 15-minute intervals to effect a complete removal of "residual" milk. For this reason, the data from the first four hourly milkings were purposely deleted when considering secretion rates.

The striking pattern of the fat content of the milk with progress of the hourly milkings, in which fat rose from a below-normal level in the early part to significantly above normal in the latter part of the trial, is not explainable on the basis of present knowledge.

The variations from hour to hour in fat per cent and the saponification numbers, as well as in the nitrogen partition and lactose content of samples analyzed less frequently, leave little doubt that there is great variation in milk composition from hour to hour and that the milk collected after a greater time interval between milkings is more constant in composition because of the fact that hourly random variability is being averaged out. There is no evidence to date to show that oxytocin treatment might increase the variability of the components of milk examined. An examination of the milks removed at hourly intervals indicates that they differ somewhat from milks obtained in a normal

manner at longer intervals. The fat content did not vary from portion to portion in any specific manner as in normal milk, and the change from the first drawn to last drawn was but slight *(10)*. For this reason, even though there may have been slight differences in degrees of evacuation or of stripping out of the ejected milk, these inequities do not explain hourly variation in butterfat per cent.

There was a much greater hourly variation in fat secretion than in milk yield. It was in only three 10-hour periods of the 156-hour trial that the relative deviations for milk production were greater than those found for fat per cent. In two of these periods, two preparations of oxytocin were used alternately. It was thought that they were of equal potency, but the erratic response in milk yields in these periods prompted a re-examination of their potency. One preparation was found to contain twice the concentration of oxytocin that it was first thought to contain. Greater yields were obtained with the more concentrated solution. In these periods, there was no apparent relationship of high yield and high fat content, thus adding further evidence that the milk obtained at hourly intervals with oxytocin does not vary within portions drawn at one milking but may vary greatly from hour to hour for reasons yet unknown.

When all of the complementary milk is obtained at each hour, any variation in butterfat percentage must arise from variation in synthesis or in transport rates between the blood stream and the lumen of the alveoli. This phenomenon of greatly varying fat secretion within a more constant volume of milk, along with the observation of the variation in nitrogen partition and lactose content, would appear to be as important in explaining day-to-day differences in fat per cent as are variations in the amount of fat-rich complementary milk not ejected or removed at each milking.

The great range of fluctuation in the saponification numbers shows that the composition of the butterfat varies from one hour to the next. But the lack of any definite trend makes it impossible to explain the cause of the variations. The correlation between the butterfat percentage and the saponification number in the four animals ranging from 0.12 to 0.64 indicates that there may be some positive relationship, but more work will be required before definite conclusions can be made.

The fluctuations of the protein and lactose show no detectable trend and, therefore, again it is not possible to formulate any theory as to the reason for the fluctuations. It appears that secretion of the various constituents is very irregular and independent of other constituents over short intervals but tends to be quite regular because of the mixing when milk is removed at much longer intervals.

The tendency of the proteins to be lower during the experimental period seems to indicate that as the interval is lengthened there is a pile-up of protein, which is not true for lactose and butterfat.

SUMMARY

Milking hourly with oxytocin brought about increases in the rates of secretion of several milk constituents, particularly during the latter part of the trials.

The fat secretion rate was particularly high after an early period of low secretion. It would appear that several milk constituents are independently formed in, or transported to, the lumen of the alveoli as seen in the independent hourly variation of total milk, milk fat (content and composition), the members in the nitrogen partition fraction, and lactose. Although most of these fractions fluctuated closely about a mean value, others fluctuated more widely and, therefore, did not closely approach the mean value in a 12-hour sample because of hourly random variability. Only total milk yields and butterfat content formed a definite time-production pattern related to the experimental routine, and these patterns were considerably different from one another. When the intervals between milkings were increased step-wise up to 16 hours, fat secretion was considerably above the rate seen in hourly milkings. Total milk secretion rate was not changed from the hourly rate until the intervals reached 16 hours, when the rate was still above the pre-experimental rate.

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THE EFFECT OF FEEDING SOYBEANS ON BLOOD PLASMA CAROTENE AND VITAMIN A OF DAIRY CALVES[1, 2]

M. F. ELLMORE[3] AND J. C. SHAW
Dairy Department
Maryland Agricultural Experiment Station
College Park

Shaw *et al. (2, 3, 4)* reported a vitamin A deficiency in newborn calves, the dams of which had received timothy hay and a concentrate mixture containing 30% ground raw soybeans. The feeding of 30% ground raw soybeans to young growing calves which were receiving 32γ of carotene per pound of body weight per day resulted in a marked depression of the blood plasma vitamin A and carotene. Squibb *et al. (5)* fed soybeans to cows and reported a depression in plasma carotene but only a slight depression in plasma vitamin A. However, these animals received relatively large and varying amounts of carotene, and no attempt was made to determine the actual carotene intake.

The level of plasma vitamin A observed by Shaw *et al. (3, 4)* on calves receiving a high intake of soybeans was considerably below what would be considered adequate on the basis of work by Moore *et al. (1)*. The studies reported herein were designed to establish the approximate carotene intake necessary to maintain a level of plasma vitamin A that could be considered safe when soybeans constitute a large percentage of the ration. Observations also were made on the influence upon plasma carotene and vitamin A of the feeding of roasted soybeans, soybeans plus thyroprotein, and soybeans when vitamin A was fed in the ester form.

EXPERIMENTAL PROCEDURE

Twenty-two calves (18 Holsteins, three Ayrshires, and one Jersey) approximately 3 months of age were used in this study. The procedure followed was to deplete the calves of vitamin A on a basal concentrate ration low in carotene *(1)* until the plasma vitamin A levels fell to approximately 4 mg. %. The concentrate mixtures are shown in Table 1. The length of time required to deplete the calves of Vitamin A varied from 50 to 80 days. One pound of nonfat dry milk solids was fed to each calf daily for the duration of the experiment. Clean wood shavings were used for bedding and were kept in the feed box at all times as the source of roughage. D-activated plant sterol was included in all rations. At the

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[3] Now at Virginia Polytechnic Institute, Blacksburg.

end of the depletion period for each calf, the animal was assigned to one of six groups as noted in Table 2, two calves being used as controls and two as test animals in each group. Groups I and IV had the same controls.

TABLE 1

Composition of concentrate mixtures in pounds

Ingredients	Basal ration[a]	A	B	C
Barley	240	215	250	250
Oats	180	250	250	250
Wheat bran	180	200	200	200
Linseed meal	60	300		
Cottonseed oil		35		
Raw soybeans			300	
Roasted soybeans				300
Salt	8	10	10	10
Plant sterol	25 g.	25 g.	25 g.	25 g.

[a] Moore *et al. (1).*

At the end of the depletion period the test animals in Groups I, II, and III received concentrate mixture B containing 30% ground raw soybeans. The control animals in each group received concentrate mixture A containing cottonseed oil. Calves in Groups I, II, and III received 32, 48, and 64γ of carotene, respectively, per day per pound of body weight.

In Group IV, the test animals received concentrate mixture C at the end of the depletion period. This mixture was identical to mixture B except that the soybeans were held at 100° C. for 30 minutes. The control animals used in Group IV were the same animals which were used in Group 1.

In Group V all of the animals received at the end of the depletion period a concentrate mixture B containing ground raw soybeans. At this time 1 g. of iodinated casein per 100 lb. of body weight per day was fed to the test animals, whereas two animals were continued on soybeans without thyroprotein as controls.

The rations fed to Group VI were identical to those fed to Group 1 except that vitamin A was used instead of carotene. Each calf received 8γ of vitamin A ester per pound of body weight per day.

The test periods ranged from 12 to 22 weeks. The experiment was originally designed for 12-week test periods, but several trials were carried beyond this time. The duration in each case is indicated by the number of analyses, since blood samples were drawn weekly. The general procedures followed, other than those indicated above, and the analytical procedures used were the same as reported previously *(3).*

RESULTS

A summary of the data is presented in Table 2. It will be noted that at the level of 32γ (Group I) and 48γ (Group II) of carotene per pound of body weight, the soybean ration depressed the plasma vitamin A. The difference between the means at the 32γ intake was significant at the 5% level, and at the

TABLE 2

The effect of feeding soybeans upon the plasma carotene and vitamin A of calves

Groups	Concentrate rations	Calf No.	No. samples	Plasma carotene	Av.	Plasma vit. A	Av.
				(γ/100 ml.)		*(γ/100 ml.)*	
	Basal	1	22	19.10 ± 2.67[a]		8.10 ± 2.54[a]	
I		2	22	38.32 ± 12.56	28.71	11.10 ± 4.09	9.60[c]
32γ							
carotene	Soybean	5	22	21.16 ± 6.46		5.57 ± 3.25	
		6	22	26.10 ± 9.45	23.63	6.66 ± 3.27	6.12
	Basal	29	13	27.50 ± 7.75		11.07 ± 1.91	
II		30	13	37.34 ± 14.00	32.42[c]	13.27 ± 2.98	12.17[b]
48γ							
carotene	Soybean	31	13	18.64 ± 5.76		8.93 ± 2.40	
		32	13	21.03 ± 6.22	19.84	6.48 ± 2.30	7.71
	Basal	33	12	32.18 ± 12.60		11.68 ± 3.04	
III		34	12	38.15 ± 13.26	35.17	9.72 ± 4.72	10.70
64γ							
carotene	Soybean	35	12	25.10 ± 6.02		9.78 ± 1.81	
		36	12	21.53 ± 6.01	23.32	9.86 ± 1.91	9.82
		1	22	19.10 ± 2.67		8.10 ± 2.54	
IV	Basal	2	22	38.32 ± 12.56	28.71	11.10 ± 4.09	9.60[b]
32γ							
carotene	Roasted soybean	9	22	39.50 ± 15.55		5.19 ± 1.94	
		10	22	18.90 ± 6.43	29.20	4.30 ± 1.55	4.75
	Soybean	17	17	17.82 ± 10.40		8.75 ± 2.88	
V		18	17	14.89 ± 8.40	16.36	5.03 ± 1.19	6.89
32γ							
carotene	Soybean and	14	17	16.25 ± 6.36		5.86 ± 1.77	
	thyroprotein	19	17	8.53 ± 3.08	12.39	4.62 ± .03	5.24
	Basal	13	16	5.58 ± 3.44		8.30 ± 1.42	
VI		16	16	2.88 ± 1.00	4.23	8.77 ± 4.76	8.54[b]
8γ							
vit. A	Soybean	11	16	12.69 ± 7.02		5.70 ± 1.61	
		12	16	2.48 ± 1.15	7.59	4.40 ± 2.30	5.05

[a] Standard deviation.
[b] $P < 0.01$.
[c] $P < 0.05$.

48γ intake the difference was significant at the 1% level. At the higher intake of 64γ of carotene per pound of body weight there was no significant depression of the plasma vitamin A. The only significant difference between the means of the various blood plasma carotene levels occurred in Group II, which received carotene at the level of 48γ per pound of body weight.

The feeding of roasted soybeans had an even greater depressing effect upon blood plasma vitamin A than did raw soybeans, as will be noted in Group IV, in which the carotene intake was 32γ per pound of body weight. The difference between the means of the basal and the soybean pairs was significant at the 1% level.

The blood plasma vitamin A levels of the soybean-fed animals in Groups I, II, and IV (32, 48, and 32γ of carotene) were all considerably below 10γ per 100 ml. of blood. These levels would not be considered safe on the basis of work by Moore *et al. (1)*. The low plasma vitamin A levels of the soybean-fed calves

in Groups I and IV were similar to those reported by Shaw *et al. (3)* at the 32γ level of carotene intake.

The data from Group V show that thyroprotein did not modify the depressing effect of soybeans on the blood plasma vitamin A, indicating that the decrease in plasma vitamin A was not due to a possible goitrogenic effect of the soybeans. In Group VI, in which vitamin A ester was fed as the source of vitamin A, the blood plasma vitamin A was depressed markedly. The difference between the means of the two pairs is significant at the 1% level, indicating that the factor or factors responsible for the depression of plasma vitamin A exert the effect regardless of whether vitamin A is supplied as such or in the form of carotene.

It is of interest that the calves gained well and appeared vigorous and thrifty on a diet in which the roughage consisted solely of shavings. The exact age of the calves is not known, but ten grade Holstein calves which were fairly uniform in weight at the beginning of the experiment (av. 149 lb.) showed an average gain of 1.86 lb. per day during a period of 140 days.

CONCLUSIONS

In all cases in which the concentrate ration contained 30% soybeans, either cooked or raw, and the carotene intake was 32γ per pound of body weight, the plasma vitamin A levels of the calves were much lower than those in the control animals and lower than would be considered safe. The data indicate that a minimum of approximately 64γ of carotene per pound of body weight is needed to provide a safe margin for young calves when soybeans make up as much as 30% of the ration. The blood plasma vitamin A was also depressed by the feeding of soybeans when vitamin A was provided in the form of vitamin A ester. Thyroprotein did not prevent the depression of plasma vitamin A caused by the feeding of soybeans.

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AGE AT FIRST CALVING USED WITH MILK YIELD DURING FIRST LACTATION TO PREDICT LIFETIME PRODUCTION OF INDIAN CATTLE[1]

D. SUNDARESAN,[2] F. E. ELDRIDGE, AND F. W. ATKESON
Department of Dairy Husbandry, Kansas State College, Manhattan

India has 176 million head of cattle (1950), one quarter of the world's cattle population, far more than needed for economical production of milk and draft power. Unprofitable cattle utilize too much of the available feed supply. If these unproductive cattle were culled, better environment and feed could be provided for those which have economic value. However, the rate of improvement of the cattle population would be a direct reflection of the accuracy of the culling. To bring about the most rapid increase in the productive potentialities of the cattle, breeding and selection methods need to be developed which are applicable to the Indian breeds in their native country. The use of such methods, coupled with educational and political programs for overcoming backward practices now prevalent in cattle husbandry, should tend to alleviate some of the conditions which for years have blocked cattle improvement.

One of the few places in India where efforts have been made to improve cattle husbandry from the standpoint of breeding and selection, as well as nutrition and management, is the Allahabad Agricultural Institute, a Christian college established in 1910. The long history of dairying at this institute has been summarized in several reports by Hutchinson and Joshi *(4)*, Schneider *(10)*, Rathore *(8)*, and Stonaker *et al. (13)*.

From 1910 until 1934, improvement of the native cattle was attempted by crossing five Indian breeds with four European breeds. Although the resulting first generation crossbreds were satisfactory, the continuation of the program presented several difficulties, such as the need for continually importing bulls of European breeds. Since 1934, two specific breeding projects have been followed: *(a)* selective breeding within the Red Sindhi breed and *(b)* crossbreeding of Red Sindhi females to Jersey males followed by backcrossing with Red Sindhi males. Genes for high milk production were expected to be introduced from the Jersey breed, and heat tolerance and disease resistance were expected to be retained through backcrossing to Red Sindhi.

All females born in the Agricultural Institute herd have been retained until they have completed at least one lactation with the exception of those that died or were nonbreeders. Culling was based on production during the first lactation,

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[2] Present address: Department of Animal Husbandry, Allahabad Agricultural Institute, Allahabad, India.

with the selection pressure depending on the number of animals retained to maintain a herd of about 100 to 150 milking cows.

Stonaker *et al. (13)* found that the first generation crossbred cattle calved at an earlier age and produced more milk in the first 305 days of their first lactation than the Red Sindhi, the differences being statistically significant. The succeeding generations of backcross cattle, up to and including those that had as little as 1/16 Jersey, showed the same trend but to a lesser extent compared with the Red Sindhi. There were also indications of greater survivability among the crossbred and backcross cattle than among the Red Sindhi cattle.

Stonaker *(12)* also studied the genetic changes in the Red Sindhi herd from 1934 to 1951, estimating probable genetic gain and heritabilities of various traits. He concluded that the selection of breeding stock and the culling of low producers had resulted in an estimated increase within this herd of 0.5 lb. of butterfat per year, or about 0.3% of the average production per year. Although he did not consider these estimates of progress discouragingly low, he believed that more rapid increases could be made in the future.

The present investigation was designed to determine how much the herd production might be increased by a selection system in which age at first calving and interval between first and second calvings were considered at the time of culling, in addition to production during the first lactation.

EXPERIMENTAL PROCEDURE

Records from the Allahabad Agricultural Institute herd for the period 1928-47 and from the Kansas State College Jersey herd for the same years were the source of data for this investigation. The Kansas State College Jersey herd was included as a convenient method for comparison. It was recognized that any similarities between the breeding programs of the two stations were coincidental.

The records of all animals that had completed at least one lactation were collected and assorted into different groups as follows:

Group	*Breeding*	*Symbol indicating breeding*
A	Red Sindhi	(RS)
B	½ Jersey ½ Red Sindhi	(½J × ½RS)
C	¼ Jersey ¾ Red Sindhi	(¼J × ¾RS)
D	½ Brown Swiss ½ Red Sindhi	(½BS × ½RS)
E	¼ Brown Swiss ¾ Red Sindhi	(¼BS × ¾RS)
F	½ Holstein ½ Red Sindhi	(½H × ½RS)
G	Murrah buffalo	(Murrah)
H	Kansas State College Jersey	(KSC-J)

For ease of reference throughout this paper the groups will be referred to by their assigned alphabetical letters, followed where necessary for clarity by the symbol describing their breeding.

Data taken from the history sheets of each animal included age at first calving, 305-day production of the first lactation, interval between first and second

TABLE 1

The coefficient of correlation between first lactation production and age at first calving

Group	Breeding	No. of pairs of observations	Average age at first calving		Average first lactation production		'r'
			Months	Std. Dev.	100 lb.	Std. Dev.	
A	RS	82	42	9.1	26	13.7	0.064
B	½J × ½RS	30	29	6.3	45	9.4	0.300
C	¼J × ¾RS	69	36	7.3	34	12.1	0.071
D	½BS × ½RS	16	42	8.0	48	12.0	0.074
E	¼BS × ¾RS	28	33	7.1	34	10.3	0.120
F	½H × ½RS	11	38	4.1	61	9.8	–0.330
G	Murrah	62	47	11.6	27	9.9	0.106
H	KSC-J	91	30	4.1	57	15.9	0.186

calving, total milk production in 7 years from date of birth, total milk production in 10 years from date of birth, and total milk production in 5 years from date of first calving. Production was measured in pounds of milk rather than pounds of fat, since most Indian dairy farms keep records in pounds of milk.

RESULTS

Interrelationship among age at first calving, first lactation production, and interval between first and second calving. The coefficient of correlation between the first lactation 305-day production and age at first calving was calculated within each group (Table 1). Actual records were used in all cases without trying to adjust for maturity, since no adequate correction factors had been devised for the Indian breeds of cattle. No statistically significant correlation was found between these two variables within any of the groups. It also was noted that in the groups with the larger numbers of cattle the correlation coefficient tended to approach zero, a fact which strengthened the conclusion that the first lactation production is independent of, or at least only weakly associated with, age at first calving.

Coefficients of correlation between age at first calving and interval between first and second calving were determined for the cows that had completed one lactation and had calved the second time (Table 2). All correlations were sta-

TABLE 2

The coefficient of correlation between interval between first and second calving and age at first calving

Group	Breeding	No. of pairs of observations	Average first calving interval	'r'
			(mo.)	
A	RS	70	16	–0.15
B	½J × ½RS	29	14	–0.21
C	¼J × ¾RS	59	16	–0.22
D	½BS × ½RS	15	15	–0.24
E	¼BS × ¾RS	26	15	0.19
F	½H × ½RS	11	14	0.36
G	Murrah	46	17	–0.25
H	KSC-J	83	13	0.03

tistically nonsignificant. Apparently the age at first calving has no effect on the breeding efficiency of the second gestation period, nor has there been any consistent trend in managing these cattle to hold younger heifers open longer before breeding them the second time.

Lifetime production as influenced by age at first calving, first lactation production, and interval between first and second calving. A true measure of the lifetime production of a dairy cow is seldom obtained, for few cows are kept in any herd until the end of their natural lives. An arbitrary period therefore must be selected, compromising between a large period of time with its consequent limitation on number of animals and a large number of animals with its lesser value in truly estimating the potential total production during a lifetime. In the early portions of the study three different periods were selected as estimates of lifetime production: *(a)* production in the 5 years following first calving (eliminating variation in age at first calving), *(b)* production up to 7 years of age, and *(c)* production up to 10 years of age. These three periods were compared in order to determine if there were any obvious differences among them as estimates of lifetime production.

TABLE 3

The coefficients of correlation between life production and age at first calving

Group	Breeding	5-yr. production following first calving		Production up to 7 years of age		Production up to 10 years of age	
		No.	'r'	No.	'r'	No.	'r'
A	RS	25	−0.425*	26	−0.544**	17	−0.662**
B	½J × ½RS	19	0.425	21	−0.095	14	0.235
C	¼J × ¾RS	23	−0.423*	23	−0.695**	14	−0.688**
D	½BS × ½RS	9	0.574	9	0.032	6	0.619
E	¼BS × ¾RS	9	−0.762*	9	−0.839**	1	——
F	½H × ½RS	9	−0.291	10	−0.286	4	−0.564
G	Murrah	11	0.393	11	−0.790**	8	−0.350
H	KSC-J	25	0.317	29	−0.051	7	−0.121
	Total	130		138		71	

* Probability less than 0.05, significant.
** Probability less than 0.01, highly significant.

The coefficients of correlation between age at first calving and each of the three estimates of lifetime production are presented in Table 3. Statistically significant negative correlations were found in groups A, C, and E, the groups containing the highest percentages of Red Sindhi breeding.

Production during the first 305-day lactation was correlated with three estimates of lifetime production and with subsequent production to 10 years of age (Table 4). The positive correlations between the first lactation production and subsequent production to 10 years of age and between first lactation production and each of the three estimates of lifetime production were significant at the 1% level of probability for groups A and H (RS and KSC-J), the pure breed groups. This correlation did not appear to exist within the crossbred groups.

TABLE 4

The coefficients of correlation between first lactation production and several estimates of lifetime or subsequent production

Group	Breeding	Production in 5 years following first calving		Production up to 7 years of age		Production up to 10 years of age		Production to 10 years of age excluding first lactation	
		(No.)	*('r')*	*(No.)*	*('r')*	*(No.)*	*('r')*	*(No.)*	*('r')*
A	RS	25	0.799**	26	0.773**	17	0.855**	17	0.785**
B	½J × ½RS	19	0.478*	21	0.260	14	0.402	14	0.281
C	¼J × ¾RS	23	0.316	23	0.220	14	0.020	14	–0.135
D	½BS × ½RS	9	0.132	9	0.668*	6	0.436	6	0.168
E	¼BS × ¾RS	9	0.139	9	0.109	1	——	1	——
F	½H × ½RS	9	0.235	10	0.568	4	0.534	4	0.414
G	Murrah	11	0.507	11	–0.415	8	0.300	8	0.077
H	KSC-J	25	0.809**	29	0.736**	7	0.907**	7	0.873**

* Probability less than 0.05, significant

** Probability less than 0.01, highly significant

In none of the breed and crossbred groups was there a significant relationship statistically between any one of the three estimates of life production and the interval between first and second calving.

Production up to 10 years of age appeared to have several advantages over the other estimates of lifetime production. First, it included the period up to first calving, which is a costly period of life and which must be considered in the total profit from a cow. Second, it included about 6 to 7 years of productive life as compared with about 3 to 4 years for the estimate up to 7 years of age. Although some other period of time might have peculiar advantages, production up to 10 years of age was selected as the basis for comparison for the remainder of the study, since it seemed to provide a more suitable goal toward which to work than the other periods.

The linear regression of production up to 10 years of age on *(a)* age at first calving and *(b)* first lactation production was determined for groups A, B, C, and H, the groups which had the largest numbers and which appeared to be the basis from which further breeding work would be done at the Allahabad Institute (Table 5). To have larger numbers and to develop a single equation which

TABLE 5

Linear regression of 10-year production on age at first calving and on first lactation production within the different breed and crossbred groups

Group	Breeding	10-yr. production in hundred pounds on age at first calving in months		10-yr. production in hundred pounds on first lactation production in hundred pounds	
		No. of pairs	'b'	No. of pairs	'b'
A	RS	17	–5.13**	17	4.32**
B	½J × ½RS	14	1.76	14	1.96
C	¼J × ¾RS	14	–3.77**	14	0.13
H	KSC-J	7	–1.57	7	5.94**

* Probability less than 0.05.
** Probability less than 0.01.

could be used for the entire herd, it was thought desirable to combine the information from groups A, B, and C if such a combination proved justifiable. The statistical technique used to determine whether such a procedure was justifiable involved the testing of three conditions: *(a)* the similarity of 10-year production of the three groups after being adjusted for group means for age at first calving and first lactation production separately, *(b)* the similarity of regression slopes, and *(c)* the use of a common regression line for each of the independent characteristics, age at first calving, and production during the first lactation *(2, 11)*. These tests indicated that the groups were not similar enough to justify combining the data into one regression equation.

It was suspected that the relatively greater uniformity within group B, the first generation crossbred group, was the reason that the three groups should not be combined. Therefore, it was decided to delete group B and test the two other groups in the same manner. The tests indicated that statistically the data from these two groups could be combined.

PREDICTION OF LIFETIME PRODUCTION

Multiple regression equations for predicting production up to 10 years of age were calculated by using the simple correlations between each combination of two among the following three variables: *(a)* age at first calving, *(b)* first lactation production, and *(c)* production up to 10 years of age. The interval between first and second calving was not included since no significant relationship was found between it and lifetime production. Two equations were developed, one for Kansas State College Jerseys, and another for groups A and C combined (RS and $\frac{1}{4}$J × $\frac{3}{4}$RS). Similar equations were not indicated for the other groups since, in each case, the correlations between these variables were not statistically significant.

KSC Jerseys. Since the relationship between production up to 10 years of age and age at first calving was not significant (Table 3), this variable was not used in a prediction equation. The simple regression equation of production up to 10 years of age on first lactation production (see Tables 4 and 5) was

$$\hat{Y} = 23.4 + 5.94X_2 \qquad r = +0.91^{**} \qquad r^2 = 0.83$$

where $\hat{Y}$ is the predicted production up to 10 years of age in hundred pounds of milk and X_2 is the first lactation production in hundred pounds of milk.

Red Sindhi and $\frac{1}{4}$ *Jersey* × $\frac{3}{4}$ *Red Sindhi.* Both age at first calving and production during the first lactation were used in calculating a multiple regression prediction equation for these combined groups of cattle (Tables 3, 4, and 5).

$$\hat{Y} = 230 - 3.24X_1 + 2.76X_2 \qquad R = 0.84^{**} \qquad R^2 = 0.71$$

where $\hat{Y}$ is the predicted production up to 10 years of age in hundred pounds of milk, X_1 is age at first calving in months, and X_2 is production during the first lactation in hundred pounds of milk. The correlation coefficient between production up to 10 years of age and first lactation production alone was +0.74, r^2 equal to 0.55. Thus, the addition of the second variable, age at first calving, increased the accuracy of prediction from 55 to 71% of the variance, a noticeable increase.

DISCUSSION

Indian cattle mature slowly. Attempts to induce early maturity in dairy cattle in India have been approached cautiously because of the opinion that production during the first few lactations might be adversely affected. The results found in this study, however, did not support this opinion since neither first lactation production nor interval between first and second calvings was affected significantly by age at first calving (Tables 1 and 2). The fact that these two variables were independent of first lactation production further supported the premise that they might be profitably included in a multiple regression equation for predicting lifetime production, provided that a significant correlation was

found between each of them and lifetime production. Since the interval between first and second calving was found to have no significant effect upon lifetime production, it was omitted from further consideration.

The absence of undesirable effects of early calving upon subsequent production agrees with the results of other recent investigations. Johansson and Hansson *(5)* found that age at first calving had no influence upon mature yield but was associated with an increase in calving interval and low initial production records. These two points differ somewhat from the results reported here. Also Gethin *(3)*, in his study of age at first calving on subsequent performance, indicated that under favorable conditions lifetime production of early calvers does not fall, or falls very little, below that of late calvers — in spite of his observations that the first lactation yields of early calvers were smaller than those of late calvers.

Several scientists have studied the relationship between some estimate of lifetime production and various measurements which could be made early in the productive life of a cow. Roth *(9)* found that the yield during the first 200 days of the first lactation accounted for 46% of the variation in production during the first 5 to 6 years after freshening. Kliesch and Bankwitz *(6)* studied two herds of European cattle in which the first 180 days production in the first lactation accounted for 29 and 52%, respectively, of the variation in the total yield during the first four lactations.

Chapman and Dickerson *(1)* studied the effect of age at first calving on several periods of production after calving and also on production up to 84 months of age. They found that although calving at an older age tended to be associated with a higher level of production after calving, the animals that calved at younger ages produced more up to 84 months of age, presumably because of their longer productive lives.

Larson *et al. (7)* found significant coefficients of correlation of 0.57 between 305-day production in the first lactation and production up to 84 months of age, and –0.27 between age at first calving and production up to 84 months of age. They also found the coefficient of correlation between age at first calving and 305-day production in the first lactation to be 0.06, indicating relative independence of these two variables. They reported that a multiple regression equation combining age at first calving and first lactation production (305 days) for predicting production up to 84 months of age accounted for 41% of the variance of this estimate of lifetime production. They pointed out, however, that the addition of age at first calving increased only slightly the accuracy of prediction from first lactation production alone.

The equation for combined groups A and C, which included a variable for age at first calving, increased the accuracy of prediction compared with prediction by first lactation production alone. The influence of age at first calving on lifetime production was marked on both the Red Sindhi breed and the first backcross of crossbreds to Red Sindhi cattle. This resulted in a greater influence of age at first calving on lifetime production than was the case with the European breeds. Most Indian cattle are bred during first heat, since they are usually more than

36 months of age at the first heat period. European cattle, on the other hand, come in heat early and a few heat periods usually have elapsed before they have reached the age of first breeding. Thus, age at first calving in European cattle is a matter of management practices, whereas in native Indian cattle the age at first calving is a consequence of age at first heat period, a physiological and probably hereditary trait.

This study substantiated the importance previously given to first lactation production as a factor for selection in the Agricultural Institute herd, but it also stressed the value of considering age at first calving as a factor in improving lifetime production in Red Sindhi or in Red Sindhi backcross cattle.

It was recognized that this study established only a measure of the phenotypic relationship of life production with age at first calving and first lactation production. The genetic relationship between these factors needs to be studied so that the influence of dams on the life production of their daughters can be established and a measure can be obtained of the genetic improvement that can be expected from selection for these factors.

SUMMARY

The objective of this study was to evaluate in Red Sindhi and Red Sindhi crossbred and backcross cattle the influence of age at first calving, first 305 days milk production in the first lactation, and interval between first and second calving on lifetime milk production. Three periods of milk production, up to 7 years of age, up to 10 years of age, and 5 years after first calving, were chosen as estimates of lifetime milk production.

Age at first calving, milk production in first 305-day lactation, and interval between first and second calving were apparently independent variables within limits of these samples.

In the Red Sindhi, ¼ Jersey–¾ Sindhi, and ¼ Brown Swiss–¾ Sindhi groups significant negative coefficients of correlation were found between 5-year milk production after first calving and age at first calving. This relationship indicated that early maturity was associated with increased milk production in these cattle, emphasizing the desirability of breeding Indian cattle for early maturity.

Milk production up to 10 years of age was considered the best estimate of lifetime production. In the Red Sindhi and ¼ Jersey–¾ Sindhi groups a large and significant negative relationship was found between 10-year production and age at first calving. In the Red Sindhi and Jersey groups a positive relationship, significant at the 1% level of probability, was found between 10-year production and first lactation production. No significant relationship existed between length of first calving interval and 10-year production in any of the breed and crossbred groups.

An equation was developed for predicting production up to 10 years of age for Red Sindhi and ¼ Jersey–¾ Sindhi as a combined group, and another equation was developed for Kansas State College Jersey cattle. For the Indian

cattle it was advantageous to use both age at first calving and first lactation production. No equations were developed for the other breed and crossbred groups because of the nonsignificant association between the 10-year production and either of the two variables. The equations are given below:

Red Sindhi and ¼ Jersey–¾ Sindhi:

$$\hat{Y} = 230 - 3.24X_1 + 2.76X_2; \; R = 0.84^{**}$$

K.S.C. Jersey:

$$\hat{Y} = 23.4 + 5.94X_2; \; r = 0.91^{**}$$

where $\hat{Y}$ is predicted 10-year production in hundred pounds of milk, X_1 is age at first calving in months, and X_2 is first lactation 305-day production in hundred pounds of milk.

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FURTHER OBSERVATIONS ON THE COMPOSITION OF GRASS SILAGE[1]

J. G. ARCHIBALD AND J. W. KUZMESKI
Massachusetts Agricultural Experiment Station, Amherst

An earlier publication *(1)* reported in considerable detail results of studies in the chemistry of grass silage as influenced by the kind of crop ensiled and the kind of preservative, if any. Since that time much additional information has been secured at this station. The present publication summarizes our work on silage composition since the study was begun in 1937. Relevant data have been incorporated from the earlier publication and from the more recent work, and correlation studies of numerous individual constituents have been made. For information on methods the reader is referred to the earlier publication. The literature on the subject is voluminous and could not be adequately covered in the space available here. For an up-to-date review the reader is referred to Barnett's recent text *(3)*.

RESULTS AND DISCUSSION

In general, although silages made from different crops and with different preservatives differed considerably in composition, only relatively few of these differences are of statistical significance. In such variable material as silage, especially when made from a mixture of crop plants, this is perhaps to be expected. The differences in chemical composition which are of statistical significance are listed below:

1. The water and protein contents of potato silage were lower than those of legume silage; the fiber and ether extract were lower and nitrogen-free extract was higher than those of all other crops investigated. In view of the starchy nature of potatoes, these differences are not surprising, even when we take into consideration the fact that the potatoes were mixed with such roughages as alfalfa hay, oat hay, and sweet corn fodder for ensiling.
2. The protein content of legume silage, as might be expected, was significantly higher than that of silage from all other crops.
3. The pH of silage made with liquid sulfur dioxide was the lowest of any; in view of the acid-forming properties of SO_2, this is easy to understand.
4. Volatile base content (an undesirable constituent above certain limits) was significantly higher in silages without preservative than in silages with preservative, notably so in comparison with the use of liquid sulfur dioxide, sodium metabisulfite, or ground wheat.
5. Butyric acid (also undesirable above certain limits) was significantly higher in untreated silage than when sodium metabisulfite was added.

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6. Lactic acid (an indicator of good quality) was significantly higher in the silages preserved with ground wheat, sodium metabisulfite, or liquid sulfur dioxide than in silages in which no preservative was used.

7. Largest amounts of carotene were present in silage preserved by the addition of sodium metabisulfite, significantly higher than with no preservative, molasses, ground wheat, citrus meal, lactic acid culture, or lactic acid culture plus salt.

8. Sugar content was much higher in silage preserved with either liquid sulfur dioxide or sodium metabisulfite than in silage preserved by certain other methods.

In general, ash, fiber, nitrogen-free extract, and ether extract were not significantly affected by method of treatment. Quality in silage as indicated by low pH, low content of volatile bases, and butyric acid, and high content of lactic acid was best attained by the addition of sodium metabisulfite, ground wheat, or hominy meal. Statistically, however, the significance of the results with hominy meal cannot be evaluated because they are from only one lot of silage. Other points of interest, which for a similar reason do not lend themselves to statistical evaluation, are described herein:

(a) The volatile base content of silage was very high when urea was added. Presumably, the urea was largely converted to ammonia; the odor of the silage suggested this, and the high pH (5.7) is further indication of it. The addition of molasses with the urea mitigated the situation somewhat, but the product was still of poor quality. Cullison *(4)* reports favorable results from the addition of urea to sweet sorghum for silage, but one is unable to judge from his report the quality of the silage from the standpoint of odor, pH, or content of the various organic acids. He does state that the untreated silage had a lower pH than that to which urea was added; this, so far as it goes, is in agreement with our findings.

(b) The use of salt either with or without a lactic acid culture resulted in silage with a rather high pH, a high content of butyric acid, and a low content of lactic acid.

(c) An excellent illustration of how a sufficient amount of suitable preservative inhibits the formation of butyric acid in silage and hence protects the quality of the final product is presented in Table 1.

TABLE 1

Effect of adding ground wheat on formation of butyric acid in silage

Ground wheat	Butyric acid content of the silage
(lb/ton of green crop)	*(%)*
100	5.8
150	1.7
200	none

Barnett *(3)* observes in several places in his recent treatise on the subject that butyric acid is formed from either lactic acid or soluble carbohydrates as

TABLE 2

Composition of silage from various crops and with various preservatives (all results except moisture and pH expressed on the dry matter basis)

By crops	No. of lots	Moisture as received	pH[a]	Carotene	Total ash	Crude protein	Crude fiber	N-free extract	Total sugars	Ether extract	Volatile bases	Total acidity	Lactic acid	Acetic acid	Butyric acid
		(%)		*(p.p.m.)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(% as NH_3)*	*(%)*	*(%)*	*(%)*	*(%)*
Mixed grasses	25	71.5 ±0.8	4.1 ±0.1	162 ±16	9.0 ±0.4	11.7 ±0.4	35.3 ±0.8	40.0 ±0.7	i.d. —	4.0 ±0.2	0.8 ±0.1	11.1 ±0.5	1.6 ±0.6	2.7 ±0.6	4.0 ±0.6
Mixed grasses and legumes	28	71.4 ±0.7	4.5 ±0.1	182 ±17	8.2 ±0.3	12.4 ±0.5	35.3 ±0.6	40.3 ±0.6	i.d. —	3.8 ±0.2	0.7 ±0.1	11.5 ±0.5	4.2 ±0.7	2.5 ±0.3	2.8 ±0.4
Legumes	5	74.6 ±1.1	4.5[b] —	150 ±42	8.5 ±0.5	16.0 ±0.6	31.6 ±1.2	39.7 ±1.4	i.d. —	4.2 ±0.2	—	not determined —			
Potatoes	4	66.2 ±2.3	4.0 ±0.1	n.d. —	7.2 ±0.7	11.5 ±1.3	22.1 ±1.4	57.7 ±2.0	n.d. —	1.5 ±0.1	—	not determined —			
By preservatives															
None	15	70.6 ±1.1	4.7 ±0.1	139 ±23	9.3 ±0.5	12.0 ±0.5	36.3 ±0.8	38.8 ±0.8	i.d. —	3.6 ±0.3	0.9 ±0.1	10.6 ±0.5	1.6 ±0.6	3.0 ±0.4	3.4 ±0.7
All preservatives	69	72.0 ±0.6	4.4 ±0.1	157 ±13	8.6 ±0.2	11.6 ±0.3	35.4 ±0.5	40.3 ±0.4	i.d. —	3.7 ±0.1	0.5 ±0.05	11.4 ±0.4	3.8 ±0.5	2.5 ±0.3	2.7 ±0.4
Molasses	28	72.5 ±1.0	4.1 ±0.2	142 ±23	8.5 ±0.4	12.4 ±0.6	35.3 ±0.8	40.0 ±0.7	0.36 ±0.16	3.4 ±0.2	0.8 ±0.1	11.8 ±0.8	3.1 ±1.0	3.4 ±0.5	3.0 ±0.8
Ground wheat	3	66.0 ±0.9	4.3 ±0.0	111 ±10	7.4 ±0.4	11.8 ±1.0	33.6 ±2.0	44.0 ±2.6	n.d. —	3.2 ±0.3	0.5 ±0.0	9.0 ±0.7	5.1 ±0.4	1.0 ±0.2	2.5 ±1.7
Corn meal	3	70.8 ±1.3	4.7 ±0.2	n.d. —	7.8 ±0.4	11.7 ±1.1	37.1 ±2.7	38.2 ±1.5	n.d. —	5.2 ±0.7	—	not determined —			
Hominy	1	69.3	4.2	135	5.1	11.5	30.1	46.9	0.80	6.5	0.5	14.2	10.1	4.2	0.3
Citrus meal	5	70.7 ±1.8	4.4 ±0.1	159 ±26	8.5 ±0.6	12.5 ±0.6	33.6 ±0.5	41.4 ±0.8	0.90 ±0.2	4.0 ±0.3	0.5 ±0.6	11.8 ±0.7	2.4 ±1.0	4.4 ±0.6	1.9 ±0.4
Phosphoric acid	7	74.8 ±1.2	n.d. —	183 ±54	9.2 ±1.0	12.4 ±1.0	35.5 ±1.4	38.6 ±1.1	n.d. —	4.3 ±0.4	—	not determined —			

TABLE 2 (concluded)

Composition of silage from various crops and with various preservatives (all results except moisture and pH expressed on the dry matter basis)

By Preservative	No. of lots	Moisture as received	pH[a]	Carotene	Total ash	Crude protein	Crude fiber	N-free extract	Total sugars	Ether extract	Volatile bases	Total acidity	Lactic acid	Acetic acid	Butyric acid
		(%)		*(p.p.m.)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(% as NH_3)*	*(%)*	*(%)*	*(%)*	*(%)*
Sulfur dioxide	4	73.1 ±2.3	3.9 ±0.1	196 ±47	7.8 ±0.6	13.8 ±0.7	32.2 ±1.0	42.5 ±0.7	4.7 ±0.2	3.7 ±0.3	0.3 ±0.07	10.2 ±0.3	5.0 ±0.8	0.1 ±0.1	3.5 ±0.5
Sodium metabisulfite	5	72.1 ±1.4	4.2 ±0.1	262 ±20	8.3 ±0.3	12.2 ±0.6	34.2 ±0.5	41.4 ±0.5	4.1 ±0.2	3.9 ±0.2	0.4 ±0.04	12.6 ±0.6	7.5 ±1.1	1.0 ±0.2	0.7 ±0.2
Salt	2	67.8	4.6	156	9.4	8.8	37.4	40.8	n.d.	3.6	0.6	10.8	2.6	1.6	3.4
Lactic acid culture	3	72.6 ±0.7	4.6 ±0.2	113 ±43	8.4 ±0.8	10.5 ±1.2	36.5 ±1.7	40.8 ±1.7	n.d. ——	3.8 ±0.4	——	——	——	——	——
Salt and lactic acid culture	6	73.0 ±2.1	4.9 ±0.1	117 ±21	9.8 ±0.8	10.7 ±1.4	39.1 ±1.9	37.2 ±2.0	n.d. n.d.	3.2 ±0.1	0.9 ±0.2	10.2 ±1.8	0.3 ±0.3	3.7 ±2.0	4.1 ±1.0
Urea	1	74.2	5.7	286	9.0	9.1	40.5	37.3	n.d.	4.2	3.8	13.1	none	5.7	6.4
Urea and molasses	1	65.7	5.3	103	7.4	9.7	37.0	42.7	n.d.	3.1	1.7	10.6	4.2	1.9	2.7

[a] Averages and standard errors determined by converting pH values to their appropriate H-ion concentrations before computation and reconverting the final result back to its pH equivalent.

[b] Only one determination.

i.d. = insufficient data

n.d. = not determined

Data on potato silages not included in the "preservative" section of the table because of their somewhat different composition.

The larger number of lots (84) in the "preservative" category than in the "crop" category (58) is due to the presence of 26 lots that could not be clearly classified by crop because of lack of sufficient information. In general, these appeared to be either mixed grasses or mixed grasses and legumes.

a final but preventable stage in silage fermentation. He points out that this occurs when there is insufficient soluble carbohydrate to further the formation of sufficient lactic acid to bring the pH of the mass down to 4.2 or less. From Table 1 it is evident that 100 lb. of ground wheat was insufficient to accomplish this result; 150 lb. greatly reduced butyric acid formation, and 200 lb. inhibited it completely. It is regretted that results from a comparable control lot are not available, but it may be noted that the butyric acid content of a similar lot in another silo with no preservative added was 6%.

Relationships between the individual constituents of silage. Correlation coefficients have been determined for the various constituents of the large number of silages, the composition of which is summarized in Table 2. A total of 91 computations of the value of r were involved in this study; of these, 24 were of significance at or beyond the 1% level (Table 3). Some interesting relationships are apparent in this table; the more important ones from the standpoint of practice in making good quality silage are described as follows:

1. High moisture content in silage is undesirable because of its association with a high content of fiber, which lowers feeding value, and with butyric acid, which is one of the substances responsible for bad odor. Furthermore, these relationships are the natural reciprocals of its negative correlation with nitrogen-free extract and lactic acid, high levels of the latter, especially, being found in silage that has a pleasing odor and has gone through a desirable type of fermentation. It is true that moisture is significantly correlated to a high degree with carotene, a highly prized constituent of any roughage. Yet even the lower levels of carotene found in silage are high enough to supply a cow's requirements. In our opinion, the objectionable odor and high fiber content of high moisture silage more than offset the advantage of high carotene levels.

2. Volatile bases (expressed as ammonia), also responsible for bad odors in silage, are very definitely associated with butyric acid and just as definitely not associated with lactic acid and low pH (high active acidity). Watson *(6)* has noted a similar situation.

3. Lactic acid is closely associated with low fiber and high nitrogen-free extract[2] and, as can be inferred from No. 2 above, is also correlated with a low level of butyric acid.

4. High protein, another desirable attribute, is also correlated with a high level of lactic acid and a low level of crude fiber.

In general, it appears that a good estimate of the quality of silage can be obtained by determining water and pH. The one exception noted in this study insofar as water content is concerned appears to be in the silages that have been preserved with sulfur dioxide either as such or in the form of sodium metabisulfite. Good quality silage has run as high as 76% water when these preservatives have been used. In substantiation of this statement, detailed analyses of five lots of silage made with sodium metabisulfite on the University farm are given in Table 4. Table 2 summarizes these results, but the detail in Table 4

[2] See reference to Barnett's observations on page 1283.

TABLE 3

Significant correlations obtained in this study

Comparisons				
Category		No. of items[a]	r =	Significance
Moisture cf	carotene	98	0.37	Highly significant[c]
	crude fiber	87	0.34	Highly significant
	N-free extract	87	−0.36	Highly significant
	ether extract	87	0.27	Significant at 1% level
	total acidity	44	0.37	Significant at 1% level
	lactic acid	44	−0.40	Significant at 1% level
	butyric acid	44	0.42	Highly significant
pH cf	volatile bases	44	−0.44[b]	Highly significant
Carotene cf	ether extract	68	0.53	Very highly significant[c]
Total ash cf	N-free extract	86	−0.41	Highly significant
	ether extract	86	−0.28	Significant at 1% level
	lactic acid	42	−0.37	Highly significant
Protein cf	crude fiber	87	−0.48	Highly significant
	lactic acid	44	0.39	Significant at 1% level
Crude fiber cf	N-free extract	87	−0.49	Highly significant
	lactic acid	42	−0.50	Highly significant
N-free extract cf	ether extract	87	−0.26	Significant at 1% level
	lactic acid	42	0.58	Very highly significant
	butyric acid	42	−0.44	Highly significant
Ether extract cf	acetic acid	42	0.38	Significant at 1% level
Volatile bases cf	lactic acid	44	−0.49	Highly significant
	butyric acid	44	0.63	Very highly significant
Total acidity cf	acetic acid	44	0.55	Very highly significant
Lactic acid cf	butyric acid	44	−0.65	Very highly significant

[a] Variation in the number of items is a matter of available data.

[b] In the sense of lower pH (increasing acidity), determined by converting pH values to their appropriate H-ion concentrations before computation and reconverting the final result back to its pH equivalent.

Formula used for computing r: $r = \dfrac{\Sigma xy}{\sqrt{(\Sigma x^2)\ (\Sigma y^2)}}$

in which x represents the variation from the mean of a single item in the "dependent variable" group of values, and y the same variation in the "independent" variable group.

Significance has been determined by reference to the table of values for this purpose given by Snedecor *(5)*.

[c] Since significance at the 1% level is generally accepted by scientists as a reliable indication that the results in question are not due to chance, it has not been thought necessary to consult tables giving the appropriate values for 0.1% and 0.01% levels of significance. The terms "highly significant" and "very highly significant" in the tabulations given above simply mean that the values so labelled are respectively somewhat beyond and away beyond the point of 1% significance as given by Snedecor.

illustrates the point more clearly. Attention is drawn especially to the low values for volatile bases and butyric acid and to the high values for lactic acid and sugar. The relatively high value for sugar is similar to that reported in an earlier paper *(2)* for silage preserved with liquid sulfur dioxide. It seems that this type of preservative protects the sugar in the crop from maximum breakdown by fermentation. The average percentage of sugar in the green crops from which the five lots of silage reported in Table 4 were made was 8.1% on the dry matter basis.

TABLE 4

Analyses of silage preserved with sodium metabisulfite

Lot No.	Moisture as sampled	pH	Carotene	Total ash	Protein	Crude fiber	N-free extract	Total sugars	Ether extract	Volatile bases	Total acidity	Lactic acid	Acetic acid	Butyric acid
	(%)		*p.p.m.*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(% as NH_3)*	*(%)*	*(%)*	*(%)*	*(%)*
1	76.3	4.5	284	9.1	11.8	34.7	39.9	4.8	4.5	0.29	10.5	3.6	0.7	1.4
2	74.3	4.1	194	7.3	10.5	35.8	42.8	4.1	3.6	0.35	13.8	7.8	0.9	0.7
3	70.8	4.1	313	8.4	13.0	32.6	41.8	3.8	4.2	0.34	13.7	8.1	1.4	0.3
4	70.8	4.1	243	8.4	11.6	34.3	42.0	4.3	3.7	0.31	12.1	8.2	0.5	0.8
5	68.3	4.3	274	8.5	13.9	33.4	40.5	3.4	3.7	0.50	13.1	10.0	1.4	0.4

SUMMARY

Results of analyses of a large number of samples of grass silage accumulated in connection with our studies from 1937 to 1953 have been compiled and correlated and are summarized herein.

Use of preservatives in general lowered significantly the volatile base and butyric acid content of silage and significantly raised the content of lactic acid.

Quality in silage, as indicated by low pH, low content of volatile bases and butyric acid, and high content of lactic acid, was best attained by the addition of sodium metabisulfite, ground wheat, or hominy meal.

Sugar content was much higher in silage preserved with either liquid sulfur dioxide or sodium metabisulfite than in silage preserved by any other method.

A study of correlation coefficients indicates that a good estimate of the quality of silage can be obtained by determining the water content and the pH.

The excellent quality of silage preserved with sodium metabisulfite is shown in considerable detail.

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THERMAL DEATH TIME STUDIES OF *BRUCELLA ABORTUS* IN MILK[1]

F. R. KRONENWETT,[2] S. A. LEAR, AND H. J. METZGER[3]
Departments of Dairy Industry and Agricultural Biochemistry
Rutgers University, New Brunswick, N. J.

The efficacy of milk pasteurization in destroying *Brucella abortus* has been studied by numerous investigators for the past quarter century. Their general conclusions were that the commercial pasteurization standard of 30 minutes at 61.7° C. was sufficient to destroy this organism.

Evans *(12)*, working with *Br. abortus* and *Br. abortus* var. *lipolyticus*, reported they were killed in 30 minutes at 52° C. and in 30 seconds at 63° C. The conclusions of Proescholdt *(24)* in 1926 were that the German low temperature holding method of pasteurization assured complete inactivation of the Brucella organisms. This method of commercial pasteurization required a holding period of 30 minutes at 60 to 63° C.

Park *(23)*, employing a concentration of five thousand billion organisms per milliliter of milk (5×10^{12}), stated that *Br. abortus* was destroyed in 7½ to 10 minutes at 60° C., in 5 to 7½ minutes at 61.1° C., and in 5 minutes at 62.8° C. In a series of papers, Carpenter and Boak *(4, 7, 8, 9)* found that the organism was destroyed in 15 minutes at 60° C., in 15 minutes at 61.1° C., in 10 minutes at 62.8° C., and in 30 to 60 seconds at 71.1° C.

Zeller and Wedemann *(28)*, employing a commercial Ahlborn pasteurizer, found no viable Brucella cells after heating from 60 to 63° C. for 10 minutes. Bartram *(3)*, however, found that *Br. abortus* was not destroyed in 20 minutes at 60° C. or in 30 minutes at 61.1° C. Seelemann and Sieg *(26)* stated that the majority of strains of 48-hour cultures of *Br. abortus* were killed in 1 to 1½ minutes at 62° C. Some strains which were heated in milk and isolated in litmus milk exhibited erratic results ("Sprungswerte") in their thermal death times. Growth would be present for a series of tubes, followed by tubes with no viable growth, and then a tube would show growth of the organism. These heat resistant cells were few in number and exhibited growth only in the litmus milk tubes and not on agar plates.

During the past decade the trend has been to pasteurize milk with the high-temperature short-time (HTST) process, in which the milk is held for 15 seconds at 71.7° C. Unfortunately, very few investigations have been carried out at these higher temperatures with *Br. abortus*. Seelemann *(25)*, working with a German HTST unit, found that a mixed culture of *Br. abortus* and *Mycobac-*

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[2] Submitted by F. R. Kronenwett in partial fulfillment of the requirements for the Ph.D. degree at Rutgers University.

[3] Agricultural Biochemistry Department.

terium tuberculosis was destroyed at 84 to 85° C. on instantaneous heating and in 13.6 seconds at 79 to 80° C.

At the present time, market milk supplies are being pasteurized almost exclusively by either the low temperature holding method or the HTST process. Many of the larger companies have utilized the higher temperature process primarily because of the increased capacity and economy of operation. From a bacteriological viewpoint, however, it is generally accepted that this process frequently yields a higher thermoduric bacteria count. A scientific explanation for this apparent difference in the relative destruction of the thermoduric organisms was presented by Ball *(2)* in 1943.

Ball was the first to offer a sound basic idea to explain the variation in the relative bacterial destruction by these two commercial processes. He believed the rate of heat inactivation or z value for the thermoduric bacteria was greater than the slope of the pasteurization curve given by these processes. By definition, the z value is the slope of the thermal death time curve, expressed in degrees of temperature, over one log cycle on semilogarithmic paper. Time is plotted logarithmically on the ordinate and temperature on the abscissa. On the basis of time and temperature requirements for the two methods of pasteurization, the slope of the pasteurization curve is 4.85° C. Ball claimed that bacteria which had lethal values close to 30 minutes at 61.7° C. and z value greater than 4.85° C. would survive the HTST pasteurization treatment at 71.7° C. for 15 seconds.

Olson *et al. (22)*, working with different strains of coliform bacteria, found that Ball's explanation held true. The z value for these cultures exceeded the pasteurization curve from 0.7 to 2.4° C. Variation in the z value was reported to be due to the temperature of incubation, age of culture, and the strain of organism. From a survey of the literature, Ball reported that the z value for *Br. abortus* was 8.0° C. He indicated that these data were not mathematically acceptable for plotting thermal death time curves since they represented a composite of several investigations.

Variables, such as age of culture and method of heat treatment, that directly affect the z value were not held constant during these experiments. Since these data implied that the Brucella organism could survive high temperature pasteurization, Foster *et al. (13)* decided to study the thermal death time curve for this organism in milk at higher temperatures. They found the z value to be 5.4° C. over the temperature range of 61.5 to 72.0° C. with a 96-hour culture of strain 2308 at a concentration of 3×10^5 cells per milliliter of milk. This value was decidedly lower than Ball's, but it still exceeded the z value for the pasteurization curve. They reported that the lethal value of their strain was 23 minutes at 61.5° C. and 14 seconds at 72.0° C. when preheating and cooling time were considered instantaneous. Since these data indicated that the lethal values may exceed the pasteurization curve in the HTST range, this study was started.

It was the objective of this investigation to establish thermal death time curves for several strains of *Br. abortus* within the temperature range of the two pasteurization processes employed for market milk at the present time.

EXPERIMENTAL PROCEDURE

Large volumes of raw milk were obtained under aseptic conditions with a modified DeLaval Sterling milking machine. A glass wool filter was placed in the vacuum line to prevent air-borne contamination when the unit was disconnected from the stanchion line. The milk was withdrawn from the milker into a sterile flask which rested in an ice water bath and stored in a refrigerator at 4° C. until needed.

Eight strains[4] of *Br. abortus* were used in this study. These were strains 19, 659, 2016, 2308, 3237, McComb, Thompson, and Woodward. Strain 2308 was the second transfer on potato dextrose agar from a culture recently isolated from an infected cow. Strains 19, 2016, 3237, McComb, Thompson, and Woodward were received as lyophilized cultures. Strain 659 was received in the lyophilized state, having been isolated in 1944. It had been carried in guinea pigs for over a year and lyophilized in 1946.

Strain 2308 was subcultured on tryptose agar *(11)* in Blake bottles for 96 hours in an atmosphere containing approximately 10% CO_2. The culture was washed off with milk phosphate buffer, prepared according to Verwey *(27)*, and 0.2 ml. was pipetted into sterile lyophilizing tubes. The tubes were shell frozen by rotation in a dry ice Methyl "Cellosolve" bath. The frozen tubes were placed on the lyophilizer and vacuum distilled at a pressure of 50μ or less. After complete desiccation, which usually required 45 to 60 minutes, the tubes were sealed off under vacuum. They were packed and stored in the refrigerator at –27.8° C. until needed.

The other seven strains were processed in the same manner except that a 1% peptone solution was employed as the substrate for lyophilizing.

When the culture was to be reconstituted, the lyophilized tube was scored with a file, wiped with alcohol, placed in a sterile cotton pad, and broken. The culture was rehydrated with 3 ml. of physiological saline, inoculated into three Blake bottles and incubated for the desired time. After incubation, these cultures were washed with physiological saline and standardized to a definite concentration in a Bauer and Pfalz Model B Photometer. The 96-hour culture of strain 2308 was standardized against a standard suspension of *Br. abortus* strain 19, obtained from the U. S. Department of Agriculture. The standard suspension contained twelve thousand million cells (1.2×10^{10}) per milliliter; the culture of 2308 was adjusted to contain one hundred twenty million cells (1.2×10^{8}) per milliliter. Gram stains were made of the cultures to check for contamination.

In the 48-hour cultures of all strains the method of subculturing was altered. The 96-hour culture, grown from the reconstituted lyophilized culture, was transferred and incubated at 37° C. for 48 hours in an atmosphere containing approximately 10% CO_2. Washed suspensions of this culture were standardized against

[4] All strains, with the exception of 659, were obtained from the Animal Disease Center, U. S. Department of Agriculture, at Beltsville, Maryland. Strain 659 was isolated from our college herd.

a constant turbidity standard prepared according to Kolmer *et al. (18)*. The standard was prepared by mixing 9.1 ml. of 1% H_2SO_4 with 0.9 ml. of 2% $BaCl_2$. The cell suspension was adjusted to contain either twenty-five thousand million cells (2.5×10^{10}) or fifty thousand million cells (5.0×10^{10}) per milliliter. Viable cell counts were made for each trial by means of 1% peptone in the dilution blanks. Huddleson's method *(16)*, incorporating a 1/700,000 dilution of crystal violet in tryptose agar, was used as the plating medium.

Identification of the organisms as *Br. abortus* species was carried out with second transfers from the reconstituted lyophilized cultures. Colonial morphology, Gram stain, and cellular morphology procedures were conducted in accordance with the *Manual of Methods for Pure Culture Study of Bacteria (10)*. The colonial characteristics were also observed by means of the oblique light technique given by Mingle and Manthei *(21)*. The slide agglutination test was carried out with a specific Brucella positive antisera. The urease activity was conducted according to the modified procedure of Hoyer *(15)* and was a semiquantitative test. The tests for bacteriostatic action of dyes and for hydrogen sulfide were performed as described by Huddleson *(16)*. The acriflavine spot test was followed as given by Braun and Bonestell *(5)*. The saline stability of a culture suspension consisted of making a physiological saline suspension of a 48-hour culture and incubating it for 24 hours at 37° C. If the culture was stable, no flocculation or settling out of the cells occurred.

Determination of the thermal death time for the organisms was carried out with equipment previously described by Foster *et al. (13)*. The sampling mechanism was automatically controlled by an electrical timing device that actuated solenoid valves on the vacuum line. This device was made up of two synchronized electric motors having 1 r.p.m. and 60 r.p.m. By utilizing these motors individually in combination with a stepping switch, electrical impulses were employed to actuate the solenoid valves. Time sequences could be selected with a selector switch so that individual samples could be taken at 1-second or 1-minute intervals. The sampling series could be spaced over a period of 1 to 43 seconds or minutes.

The mixing efficiency of the equipment was checked by using methylene blue as the inoculum. It was found that complete blending of the inoculum occurred within 2 seconds from the time of injection.

The milk was flash heated to 80° C. and cooled to the predetermined temperature, which was controlled to within ±0.1° C. The temperature of the milk was measured by a 76-mm. immersion thermometer which had been checked against a Bureau of Standards certified thermometer at operating temperatures. Determination of the inactivation point of the Brucella was conducted by injecting the culture into the milk and sampling at predetermined time intervals. The withdrawn samples were cooled instantly, since the sample tubes rested in an ice water bath. The tubes were plugged with sterile cotton and placed in the refrigerator over night. The next morning 0.2 ml. of cream was plated in duplicate on tryptose agar with the recommended crystal violet. The cream was spread evenly over the surface of the agar by means of an enlarged oblong platinum needle. These plates were incubated at 37° C. under an atmosphere

containing approximately 10% CO_2. The plates were examined after 5 days incubation for growth of typical pearl-like colonies. Further identification was made by making a Gram stain.

RESULTS AND DISCUSSION

The results of the morphological and biochemical tests, when compared with Bergey *(6)*, Henry *(14)*, and Huddleson *et al. (17)*, identified all strains as smooth variants of *Br. abortus*. Strain variations in urease activity, rate of H_2S production, and colony diameter were evident but not to any large extent.

TABLE 1
Time-temperature requirements for inactivation of Brucella abortus in milk

Strains	Age of culture	Viable cells per ml. milk	Holding temp.	Thermal death time
	(hr.)	*(millions)*	*(° C.)*	*(seconds)*
19	48	200	64.0	32-34
19	48	200	64.4	32-34
19	48	200	64.6	26-29
19	48	200	64.9	24
19	48	200	65.4	18
19	48	200	65.9	12
659	48	200	65.1	35-38
659	48	200	65.3	26-30
659	48	200	66.0	18-31
659	48	200	66.7	14
659	48	200	67.0	13
2016	48	200	64.3	70-75
2016	48	200	65.5	38-40
2016	48	200	66.6	20-25
2308	96	0.3	61.5	95-100
2308	96	0.3	63.3	33
2308	96	0.3	66.5	9
2308	48	100	61.6	150-180
2308	48	100	65.0	28-30
2308	48	100	66.7	16-18
2308	48	100	66.8	10-12
3237	48	100	63.4	36-38
3237	48	100	64.5	22-25
3237	48	100	64.8	16-18
3237	48	100	66.5	9
McComb	48	100	61.5	240-270
McComb	48	100	65.0	32-34
McComb	48	100	65.6	24-26
McComb	48	100	66.0	22-24
McComb	48	100	66.5	12-14
Thompson	48	200	61.5	150-180
Thompson	48	200	63.5	74-79
Thompson	48	200	64.3	53
Thompson	48	200	65.1	35-37
Thompson	48	200	65.4	26-28
Thompson	48	200	66.3	20-22
Woodward	48	200	66.6	24-26
Woodward	48	200	67.2	16-20
Woodward	48	200	67.8	14-16

The data given in Table 1 are the summation of over 225 experimental trials. Only the pertinent data are listed since the remaining data, although significant and corroborative, are not applicable to rate of total destruction experiments.

The thermal death time curves of eight strains of *Br. abortus* were determined and their z values obtained. The variables, strain, concentration of cells and age of culture that had a direct effect upon the rate of inactivation were held constant during the individual experiments. Five strains were studied at an age of 48 hours in a concentration of 2.0×10^8 cells per milliliter. Three strains were studied at an age of 48 hours in a concentration of 1.0×10^8 cells per milliliter. One strain was studied at 48 and 96 hours in a concentration of 1.0×10^8 cells per milliliter for the former and 3×10^5 cells per milliliter for the latter. A summation of the results is given in Table 2.

TABLE 2

Experimental z values for Brucella abortus

Strain	Age of culture	Cone/ml of milk	z value
	(hr.)	*(millions)*	*(° C.)*
19	48	200	4.3
659	48	200	4.3
2016	48	200	4.8
2308	96	0.3	4.8
2308	48	100	4.7
3237	48	100	4.7
McComb	48	100	4.3
Thompson	48	200	4.8
Woodward	48	200	4.8

The experimental range for the z value was from 4.3 to 4.8° C. The z values for the 48-hour and 96-hour cultures of strain 2308 differed only by 0.1° C., being 4.8° C. for the 4-day culture (Figure 1) and 4.7° C. for the 2-day culture (Figure 2).

Experimentally this slight difference is not considered significant, since the temperature control during the experiment was accurate only to 0.1° C. These data indicate that the age of culture and concentration of organism do not affect the z value beyond the limits of experimental error. However, the strain of the organism does affect the z value, as shown in Figures 2 and 3.

In Figure 2, strain 3237 exhibits the lowest lethal values over the temperature range of 62 to 68° C. However, at higher temperatures the McComb strain would have a lower lethal value since its z value is less than that of strain 3237. Here we have the effect of strain upon z value and lethal value.

There are similar curves for additional strains in Figure 3 wherein the cell concentration has been doubled. The displacement of the curves is upward in the case of strains 2016 and Woodward. These data indicate that this is due to the increased cell concentration and the effect of strain. Strain 19 exhibited the lowest lethal values and strain 2016 and Woodward were the most heat resistant. It is interesting to note here that the Woodward and 2016 strains appear to have the same z value and almost identical lethal values at 66.6° C.

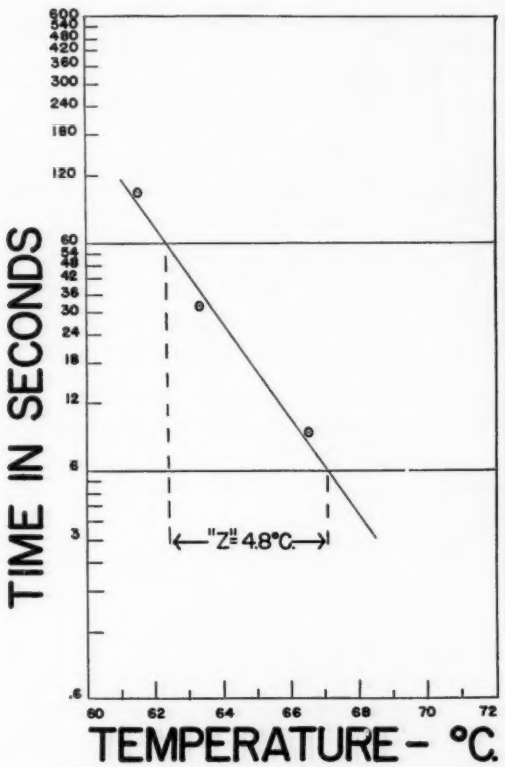

FIG. 1. Thermal death time curve: 96 hour culture of *Brucella abortus*-2308. Viable cell concentration 3×10^5 cells/ml of milk.

From the curves in Figure 2 it can be stated that the three strains were destroyed between 6 and 17 seconds at 67° C. and between 2 and 6 minutes at 61° C. Similarly, by examining Figure 3 it can be said that the five strains were destroyed between 6 and 24 seconds at 67° C. and between 4 and 6 minutes at 61° C. These lethal values at the lower temperatures near 60° C. are in agreement with the values determined by previous workers *(12, 23, 26)*.

Strain 19, having a low degree of virulence, is employed as a calfhood vaccine against brucellosis by the U. S. Department of Agriculture. From Figures 2 and 3 it can be seen that strain 3237 and strain 19 have the lowest lethal values, whereas the more virulent organisms have higher lethal values. Assuming that the lower lethal values are associated with degree of virulence, strain 3237 may warrant investigation for use as a vaccine.

In Figure 4 a comparison is shown between the z value and lethal values of *Br. abortus* 2016 to the pasteurization and phosphatase inactivation curves for milk. Strain 2016 was the most heat-resistant of the organisms studied, with

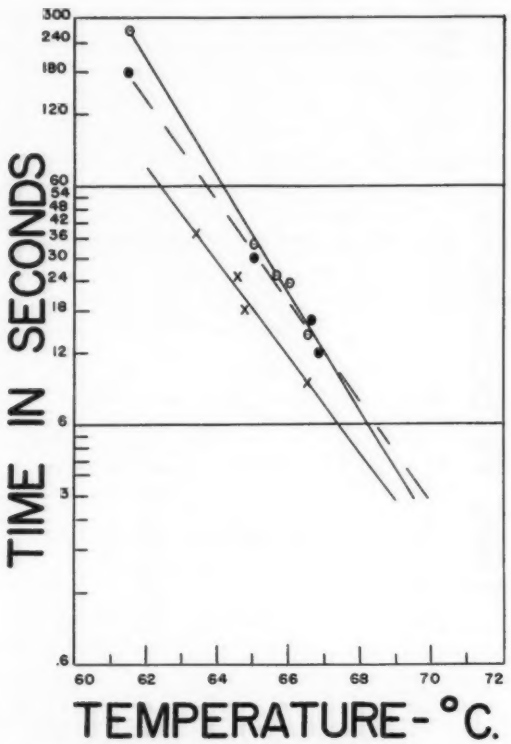

FIG. 2. Effect of strain on "z" value and lethal value of a 48-hour culture of *Brucella abortus*. Viable cell concentration 10×10^7 cells/ml of milk.

⊙——⊙ *Brucella abortus*–McComb ($z = 4.3°$ C.)
●——● *Brucella abortus*–2308 ($z = 4.7°$ C.)
×——× *Brucella abortus*–3237 ($z = 4.7°$ C.)

the exception of the Woodward strain. As stated before, these two strains appeared to have comparable lethal values from analysis of their curves in Figure 4. The curve for phosphatase inactivation was that determined by Lear and Foster *(19)* obtained by the New York City Board of Health rapid test. The slope of the pasteurization curve was taken from the latest U. S. Public Health Service recommendations *(20)* for pasteurization standards. The z value for *Br. abortus* 2016 is 4.8° C., which is essentially the same as that of the pasteurization and phosphatase inactivation curves. The significant factor from the public health viewpoint is the position of the curve on the graph. It can be seen that there is a definite margin of safety, approximately 14 minutes at 63° C. and 75 seconds at 68° C., between the pasteurization curve and the lethal values of *Br. abortus* 2016. These studies were carried out under experimental conditions of instantaneous heating and cooling so that in commercial practice an even

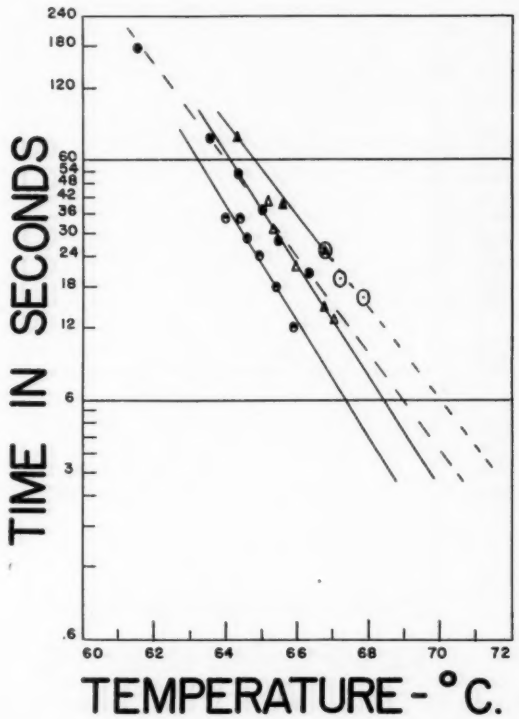

FIG. 3. Effect of strain on "z" value and lethal value of a 48-hour culture of *Brucella abortus*. Viable cell concentration 20×10^7 cells/ml of milk.

Brucella abortus-2016 ($z = 4.8°$ C.)
Brucella abortus-Woodward ($z = 4.8°$ C.)
Brucella abortus-Thompson ($z = 4.8°$ C.)
Brucella abortus-659 ($z = 4.3°$ C.)
Brucella abortus-19 ($z = 4.3°$ C.)

larger margin of safety would be present. The effect of preheating in a commercial pasteurizing unit could be expected to contribute to a major portion of the lethal value needed to inactivate the Brucella at the higher temperature.

The z value obtained by Foster *et al. (13)* for *Br. abortus* 2308 compares favorably with the current study. Although there is a divergence of 0.6° C. higher in their value, experimentally the values are compatible since it has been found in the canning industry that a variation of 1.0° C. or more for the same organism is common. However, the lethal values for 2308 in this study are decidedly lower. Since some workers *(1, 3, 4)* have also reported higher lethal values within the same range for Brucella, it is possible that Foster was working with a more heat-resistant colonial variant of Strain 2308.

It must be remembered, however, that in a market milk supply the number

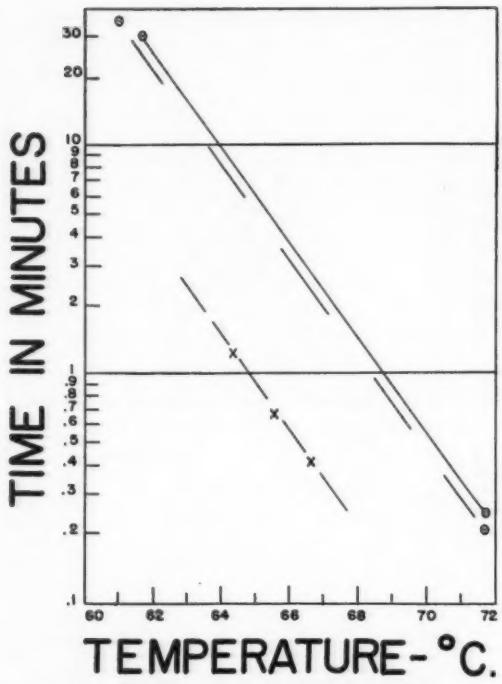

FIG. 4. Relationship of "z" value and lethal value of *Brucella abortus*-2016 to pasteurization and phosphatase inactivation curves for milk.

⊙————⊙ U. S. Public Health Service Pasteurization Standards (20) ($z = 4.85°$ C.)
⊙— — —⊙ Phosphatase Curve Lear & Foster (19) ($z = 4.9°$ C.)
—×—×— 48 Hour *Brucella abortus*–2016 Death Curve ($z = 4.8°$ C.)

of Brucella organisms would be decidedly lower than the number used in this experimental study. Therefore, the results of this study indicate that the present standards of pasteurization are adequate for destroying *Br. abortus*.

CONCLUSIONS

The experimental range for the z values of eight strains of *Brucella abortus* in this study was 4.3 to 4.8° C.

The thermal death times of these *Brucella abortus* strains were considerably below the pasteurization curve as recommended by the U. S. Public Health Service. The present pasteurization standard for the holding method of 30 minutes at 143° F. has a margin of safety of approximately 26 minutes. By extrapolating the thermal death time curve from 67.8 to 71.7° C., a margin of safety of approximately 12 seconds at 161° F. is indicated.

Under the conditions of this study, the age of culture and concentration of cells did not affect the z value of strain 2308.

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DILUTERS FOR BOVINE SEMEN. IV. FERTILITY OF BOVINE SPERMATOZOA IN HEATED HOMOGENIZED MILK AND SKIMMILK[1]

J. O. ALMQUIST, R. J. FLIPSE, AND D. L. THACKER[2]
Dairy Cattle Breeding Research Center
The Pennsylvania State University, State College

Since 1950 this laboratory has been studying the use of milk as a diluter for bull semen. The first fertility trials in 1951 and 1952 *(1, 9)* showed heated fresh homogenized milk without antibiotics to be equally as good a diluent as egg yolk–citrate containing penicillin and streptomycin. A 1952 report by several artificial breeding organizations indicated variable results from use of heated milk diluters *(6)*. During 1953 Dreher and Webb *(4)* and Flerchinger *et al. (5)* showed that heated homogenized milk with penicillin and streptomycin gave fertility results superior to those obtained with egg yolk diluters containing antibiotics, and Collins *(3)* reported equal results with the two types of diluters.

Field reports from several technicians during the first field experiment *(9)* indicated that milk diluter tended to leak from the inseminating tubes because of its low viscosity. Laboratory studies by Thacker *(8)* with gelatin, Irish moss, gum acacia, a high viscosity gum (sodium carboxymethylcellulose), and cornstarch showed that the addition of 1% cornstarch to milk diluter provided viscosity very similar to that of egg yolk–citrate (1:1).

The present field trials were undertaken to compare the fertility of bull spermatozoa diluted in heated fresh homogenized milk, heated fresh pasteurized skimmilk, and egg yolk–citrate. In one trial cornstarch was added to one of the milk diluters to determine if the resulting increase in viscosity was beneficial.

EXPERIMENTAL PROCEDURE

Although further studies of the method of heating milk for use as a bovine semen diluter are in progress, the procedure recommended is as follows:

Heat fresh homogenized milk or pasteurized skimmilk to 92° C. over water in a covered double boiler; hold between 92 and 95° C. for 10 minutes; cool; add antibiotics and semen. If an open vessel is used, follow the same heating procedure but avoid excessive heating (98° C. and over for more than 10 minutes). If milk is heated in a vessel directly over the source of heat, browning can be prevented by not heating too rapidly. Use of a thermometer is suggested. Surface scum on heated homogenized milk can be removed by decanting or filtering through a sterile gauze.

The first split sample experiment was conducted in cooperation with the First Pennsylvania Artificial Breeding Cooperative, Lewisburg, from January 6 to April 18, 1952. Homogenized milk standardized to 3.8% fat and containing

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[2] Present address: Carnation Milk Farm, Carnation, Wash.

400 U.S.P. units of activated ergosterol per quart (A.R.P.I. process) was heated over water in a covered aluminum double boiler on a commercial electric range. The 10-minute holding period was started when the milk reached 92° C., and the milk usually reached a maximum of 98-99° C. during this period. The milk was cooled rapidly to room temperature in an ice bath and used as a diluter the same morning in which it was prepared. Two double boilers were used so that 10 g. of cooking cornstarch per quart of milk could be added to one lot. The cornstarch went into solution readily by preparing a paste with a small amount of cool milk. When the milk being heated reached 92° C., the starch paste was added with constant stirring.

After cooling, 1,000 units each of penicillin and streptomycin per milliliter of diluter were added to the previously heated milks, as well as to the control diluter consisting of one part egg yolk and one part 2.9% sodium citrate dihydrate. Each of 105 semen samples from seven highly fertile Holstein bulls was divided into three portions; one portion was added to heated milk, one portion to heated milk containing cornstarch, and one portion to egg yolk–citrate. The technicians of the association were divided into three groups and supplied with semen diluted with each of the three diluters in three successive weeks. In no case was the semen diluted to contain less than 15,000,000 motile spermatozoa per milliliter of diluted semen.

A second split sample experiment was conducted at the Southeastern Pennsylvania Artificial Breeding Cooperative, Lancaster, from February 2 to April 4, 1952. Homogenized milk, pasteurized skimmilk and yolk–citrate diluters were compared, using the same experimental design as in the previously described trial. Fresh homogenized milk without added vitamin D and fresh skimmilk were obtained from a dairy plant. The milks were heated over water in a covered Pyrex glass double boiler on a laboratory hot plate with a rheostatic control switch. Ten grams of cornstarch per quart was added routinely to each of the milk diluters when they reached 92° C. Then the amount of heat was reduced so that the temperature never exceeded 95° C. during the 10-minute holding period. The heated milks were allowed to cool slowly to room temperature, antibiotics were added, and the milks were stored at 5° C. over night and warmed to room temperature the following morning just before the semen was added. Penicillin and streptomycin were added to each of the three diluters at the rate of 1,000 units of each per milliliter. In addition, the egg yolk–citrate (1:1) diluter, but not the milk diluters, contained 300 mg. % of sulfanilamide.

Seventy-two ejaculates from five Holstein and three Guernsey bulls of average to above average fertility were involved. The average rate of semen dilution was 1:70 and ranged from 1:50 for some of the samples from Guernsey bulls up to 1:150 for some of the Holstein samples. Approximately 5% of the diluted semen was used the day of collection, 75% the day after collection, and the remaining 20% the second and third days after collection.

Fertility in both experiments was based on the percentages of 60- to 90-day nonreturns for first service cows only. The data were analyzed by analysis of variance according to Snedecor *(7)*.

TABLE 1

Fertility of semen diluted with heated homogenized milk with and without cornstarch

Day semen used	Diluters					
	Milk		Milk–cornstarch[a]		Egg yolk–citrate	
	No. serv.	% N.R.	No. serv.	% N.R.	No. serv.	% N.R.
Day 1[b]	217	73.7	249	75.5	224	73.2
Day 2	1,353	76.5	1,305	74.1	1,341	76.4
Total	1,570	76.1	1,554	74.3	1,565	75.9

[a] 10 g. of cornstarch per quart.
[b] Semen used day of collection.

RESULTS

The average fertility results for the first experiment involving 4,689 first service cows are shown in Table 1. Analysis of variance of these data showed no significant differences among diluters. Table 1 also shows that with the milk and egg yolk diluters there was no difference in using the semen for insemination the day of collection or the day after. In this study 818 cows were bred with semen more than 48 hours old, but 77% of these services were to one bull. Because of the bias which would have been introduced by inclusion of such a disproportionate number of services to one bull, all data involving semen used beyond the first day after collection were discarded. Elimination of these data did not affect the average nonreturn rates for the three diluters (Table 1). The variation among bulls and among technician groups was not significant. However, one bull averaged 5% higher on yolk–citrate than on either of the milk diluters, and two bulls averaged 5 and 7% higher in favor of the milk diluters.

TABLE 2

Fertility of semen diluted with heated homogenized milk and heated pasteurized skimmilk

Breed	Diluters					
	Homogenized milk[a]		Skimmilk[a]		Egg yolk–citrate[b]	
	No. serv.	% N.R.	No. serv.	% N.R.	No. serv.	% N.R.
Holstein	2,300	65.1	2,302	68.6	2,120	66.1
Guernsey	552	65.9	571	64.8	554	69.7
Total	2,852	65.3	2,873	67.8	2,674	66.8

[a] 10 g. of cornstarch per quart.
[b] 300 mg. % of sulfanilamide.

Table 2 presents the average fertility results for the second experiment, involving 8,399 first service cows in which homogenized milk, skimmilk, and yolk–citrate diluters were compared. Analysis of variance showed that the difference among diluters was not significant. As in the first experiment the difference among bulls and among technician groups was not significant. Only one of the eight bulls showed a large difference between diluters. This bull, the lowest fertility bull in the study, showed an average increase of over 10% in favor of the milk diluters.

DISCUSSION

These results of using fresh heated homogenized milk as a diluter for bull semen substantiate the favorable fertility results of other research reported in this country *(1, 4, 5, 10)*. The similarly acceptable results obtained with fresh heated skimmilk support laboratory studies by Thacker and Almquist *(10)*, in which heated skimmilk maintained livability of bull spermatozoa approximately equal to heated homogenized milk and egg yolk–citrate. However, Bolton and Durrell *(2)* recently reported a significant difference of 4.28% in favor of yolk–citrate over pasteurized skimmilk. Although 500 units each of penicillin and streptomycin per milliliter of diluter were added, they suggested that the somewhat higher numbers of bacteria found in the skimmilk diluter may have contributed to the lower fertility obtained. The use of higher concentrations of penicillin and streptomycin in the present studies (1,000 units of each per milliliter) may have obviated this possibility.

The addition of cornstarch to increase the viscosity and ease of handling of milk diluter during insemination had no significant effect on fertility. However, the milk plus cornstarch diluter averaged 1.8% lower in nonreturn rate than the milk diluter without cornstarch. After the completion of this trial, the amount of cornstarch used routinely by the breeding association gradually was reduced from 10 to 7.5, 5, and 2.5 g. per quart of milk and finally eliminated entirely. Experience in handling milk diluter apparently overcame the early difficulty as no complaints were received from the technicians after complete removal of the cornstarch. In the light of this observation and the slight decrease in fertility, the use of cornstarch appears unwarranted and is not recommended.

Based on limited data, there was no indication of a difference in fertility of the diluters associated with age of semen. This finding is in agreement with Flerchinger *et al. (5)*, who previously reported that milk used for 3 days maintained satisfactory fertility as compared to yolk–citrate.

SUMMARY

By means of the split sample technique, two field trials were conducted to compare the fertility results obtained with fresh heated milks and egg yolk–citrate as diluters for bull semen. Penicillin and streptomycin were added routinely to all diluters.

In the first trial, 105 semen samples from seven Holstein bulls of high fertility were used to inseminate 4,689 first service cows. The average 60- to 90-day nonreturn rate was 76.1% for heated homogenized milk, 74.3% for heated homogenized milk plus cornstarch, and 75.9% for egg yolk–citrate. The differences among diluters were not statistically significant according to the analysis of variance. The addition of cornstarch to increase the viscosity of milk diluter did not improve fertility and was found to be unnecessary.

The second experiment compared heated homogenized milk and heated pasteurized skimmilk (both containing cornstarch) with the control yolk–citrate diluent. Based on inseminations to 8,399 first service cows with 72 ejaculates

from eight bulls of average or above average fertility, no significant differences in fertility were obtained. The nonreturn rate was 65.3% for heated homogenized milk, 67.8% for heated skimmilk, and 66.8% for yolk–citrate.

Based on these fertility results, it is concluded that fresh heated homogenized milk and fresh heated skimmilk can be used as practical diluting fluids for bull semen.

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DILUTERS FOR BOVINE SEMEN. V. A COMPARISON OF HEATED MILK AND EGG YOLK–CITRATE AS DILUTERS FOR SEMEN FROM BULLS OF HIGH AND LOW FERTILITY[1]

J. O. ALMQUIST

Dairy Cattle Breeding Research Center
The Pennsylvania State University, State College

Heated homogenized milk has been shown to provide fertility results equal to or better than egg yolk–buffer diluters *(1, 3, 4, 6)*, and there is evidence that it is of special value as a diluent for semen from bulls of relatively low fertility. Almquist and Thacker *(2)* and Dreher and Webb *(3)* have reported significant increases in the nonreturn rates of relatively infertile bulls when their semen was diluted with heated homogenized milk containing penicillin and streptomycin as compared to egg yolk diluters with antibiotics.

The objectives of the fertility tests reported herein were: *(a)* to study the effect of adding penicillin and streptomycin to heated milk diluter, and *(b)* to compare heated milk and egg yolk–citrate as diluters for semen from bulls of relatively high and low fertility.

EXPERIMENTAL PROCEDURE

Two split sample experiments were conducted at the Western Pennsylvania Artificial Breeding Cooperative from July 23 to October 16, 1951, and from January 6 to February 28, 1953. In each study the bulls were selected and classified as being either high or low in fertility on yolk–citrate diluter prior to the start of the trial. In both experiments the 60- to 90-day nonreturn rates for first service cows were used for statistical analysis according to Snedecor *(5)*.

Experiment 1. Twelve bulls, three Guernseys and three Holsteins of high fertility and a like number of low fertility, were selected. Semen samples were collected at weekly intervals and each sample was divided into three portions; one third was diluted with heated homogenized milk containing penicillin and streptomycin, one third with heated milk alone, and the remaining third with egg yolk–citrate–penicillin–streptomycin. The average dilution rate was 1:35 and ranged from 1:25 to 1:65. Technicians were divided into three groups and had an equal opportunity to use each diluter.

Fresh homogenized, pasteurized milk was obtained from a local dairy plant, and approximately 1.75 qt. was heated in an open 2-l. Pyrex glass beaker on a gas hot-plate. When the milk reached 92° C., the flame was reduced and the timing for the 10-minute holding period was started. In about 2 minutes the

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milk had reached 98° C. and was held at 98 to 99° C. for the remaining 8 minutes, at which time the beaker of milk was removed from the hot-plate and covered. The heated milk was allowed to cool gradually to room temperature (about 50 minutes) and transferred to sterile, covered 1-l. Pyrex glass cylinders; antibiotics were added according to the experimental design, and the milk was stored at 5° C. for use the following morning. Penicillin and streptomycin were added to the egg yolk–citrate (1:1) diluter and half of the heated milk at concentrations of 1,000 units of each per milliliter. Cornstarch, a thickening agent added to milk diluter in two other field trials *(1)*, was not used.

Experiment 2. Sixteen Guernsey and 14 Holstein bulls were used to compare heated homogenized milk with antibiotics and yolk–citrate with antibiotics. Based on the results of Experiment 1, milk without antibiotics was not tested. Eighteen of these bulls, equally divided into high and low fertility groups, were in active service on yolk–citrate during 1951 and contributed information on the difference between the diluters in relation to the levels of fertility of the bulls.

The diluters were prepared as previously described. Half of each semen sample was diluted with heated milk and half with egg yolk–citrate and shipped on alternate weeks to each of two technician groups. To avoid bias among the technicians in the selection of milk or yolk–citrate diluted semen for insemination, semen from the remaining 12 Guernsey and Holstein bulls collected on the same days as that from the 18 selected bulls of high and low fertility was diluted on the same split sample basis. Thus, the technicians could not discriminate against one diluter or the other, as all Guernsey and Holstein bull semen received was diluted with either milk or yolk–citrate. The rate of semen dilution averaged 1:50, with a range from 1:30 to 1:80.

RESULTS AND DISCUSSION

Experiment 1. The average 60- to 90-day nonreturn rates involving 144 ejaculates used to inseminate 6,670 first service cows are presented in Table 1. Analysis of variance of these data revealed significant differences ($P < 0.05$) among diluters. The mean difference between milk with antibiotics and yolk-

TABLE 1

Average fertility of semen diluted with heated homogenized milk with and without penicillin and streptomycin in Experiment 1

Breed	Diluters						No. bulls
	Milk		Milk-antibiotics		Yolk–citrate-antibiotics		
	(No. serv.)	*(% N. R.)*	*(No. serv.)*	*(% N. R.)*	*(No. serv.)*	*(% N. R.)*	
Guernsey	1,083	70.7	1,098	72.4	1,171	64.6	6
Holstein	1,080	66.1	1,160	71.4	1,078	65.4	6
Total	2,163		2,258		2,249		12
Services weighted equally		68.4		71.9		65.0	
Bulls weighted equally		66.2		72.1		64.1	

citrate with antibiotics of 8.0% in favor of milk was highly significant ($P < 0.01$). The addition of penicillin and streptomycin to milk resulted in a significant ($P < 0.05$) mean improvement of 5.9% over milk not containing antibiotics. The experimental design did not include yolk–citrate diluter without penicillin and streptomycin as a treatment. Thus, in the present study it was not possible to determine whether the addition of antibiotics to milk increases fertility to a greater or lesser degree than the addition of antibiotics to yolk–citrate diluter. The differences among bulls were not statistically significant. The interaction of bulls and diluters was highly significant, but all other first order interactions involving bulls, diluters, technician groups, and replicates were found to be not significant.

Fertility data based on 180- to 210-day nonreturns to service also were collected but they did not reveal any change in the relationships among diluters. For each diluter there was a 5% decrease in per cent nonreturns when the 60- to 90-day and 180- to 210-day nonreturn figures were compared.

The highly significant interaction of bulls and diluters indicated that the effect of the diluters was significantly different for the various bulls. The average 60- to 90-day nonreturn rates for the individual bulls on the milk and yolk–citrate diluters containing penicillin and streptomycin are shown in Table 2. Also shown is the average nonreturn rate for each bull during the 6-month pre-

TABLE 2

Comparison of milk and yolk–citrate diluted semen from bulls of relatively high and low fertility in Experiment 1

Bull	6-mo. pre-expt. yolk–citrate[a]		3-mo. expt. yolk–citrate[a]		3-mo. expt. heated milk[a]		Experimental difference (Milk–yolk)
	(No. serv.)	*(% N. R.)*	*(No. serv.)*	*(% N. R.)*	*(No. serv.)*	*(% N. R.)*	
			Relatively high fertility bulls				
H43	2,524	72	251	74	228	71	−3
H36	2,287	66	276	67	320	69	+2
H38	1,711	66	163	66	176	70	+4
G61	1,275	66	221	70	187	77	+7
G43	1,455	65	245	71	194	75	+4
G15	1,614	64	241	65	218	76	+11
Total	10,866		1,397		1,323		
Bulls weighted equally		66.5		68.8		73.0	+4.2
			Relatively low fertility bulls				
H56	1,070	60	183	57	176	75	+18
H31	1,520	58	97	57	127	77	+20
H34	1,089	58	108	62	133	68	+6
G67	396	63	119	66	147	63	−3
G53	1,726	58	179	60	164	67	+7
G42	954	57	166	52	188	73	+21
Total	6,755		852		935		
Bulls weighted equally		59.0		59.0		70.5	+11.5

[a] 1,000 units each of penicillin and streptomycin per milliliter.

experimental period on yolk–citrate with antibiotics. Based on the means weighted by bulls rather than services, it is apparent that the bulls classed as low fertility prior to the experiment showed a much greater increase in fertility when their semen was added to heated milk plus penicillin and streptomycin than the bulls classed as high fertility. The average difference in favor of milk shown in Table 2 was 4.2% for the six high fertility bulls and 11.5% for the six low fertility bulls. Thus, the major portion of the 8.0% over-all improvement between these two diluters shown in Table 1 is accounted for by the response of the relatively infertile group of bulls. Only one of the six high fertility bulls exceeded the over-all difference of 8.0%, whereas three of the six low fertility bulls exceeded this difference and each by a substantial margin.

A highly significant negative correlation ($r = -0.72$) was obtained between the nonreturn rates for the bulls in the 6-month pre-experimental period and the increase in nonreturn rates during the experimental period when heated milk was used instead of yolk–citrate, both of which contained antibiotics. It is recognized that part of the difference in response between high and low fertility bulls may have been due to the different effects of chance variation on individual bull data during the pre-experimental and experimental periods.

Milk diluter without antibiotics averaged 70.7% for the six high fertility bulls and 63.3% for the six low fertility bulls, or 1.9% and 4.3% higher in nonreturn rates than egg yolk–citrate containing antibiotics and 2.3% and 7.2% lower in nonreturn rates than heated milk with antibiotics.

Although about 90% of the diluted semen from all bulls in the stud was used the day after collection, it was possible to obtain some data on the effect of age of semen upon the nonreturn rates for semen diluted with milk and yolk–citrate. Semen collections were made daily, except Sundays, and it occurred that five of the bulls selected for the present study were collected on Saturdays and the semen used the following two days. The five bulls whose semen was used on weekends included two bulls of high fertility (H38 and G15) and three bulls of low fertility (H34, G53, and G67). Table 3 shows the average fertility of the diluted semen from these bulls used for insemination the first and second days after collection. There was no difference in the average decrease in nonreturn rates for the milk and yolk–citrate diluted semen used the second day after collection.

TABLE 3

Effect of age of semen upon fertility in heated milk and egg yolk–citrate diluters (based on five of bulls in Experiment 1)

Day of use	Milk		Milk–antibiotics		Yolk–citrate-antibiotics	
	(No. serv.)	*(% N. R.)*[a]	*(No. serv.)*	*(% N. R.)*[a]	*(No. serv.)*	*(% N. R.)*[a]
Day 2[b]	447	70	444	74	429	64
Day 3	427	68	419	70	412	59
Total	874	69	863	72	841	62

[a] Bulls weighted equally.
[b] Semen used day after collection.

Experiment 2. Because of the indication that milk was superior to yolk–citrate for diluting semen from low fertility bulls in Experiment 1, a second experiment was designed in 1952.

The average fertility results for the 30 bulls and 240 semen samples are shown in Table 4. Analysis of variance involving 8,400 first service cows showed that the average difference of 7.5% in favor of heated milk diluter was highly significant. No attempt was made to remove the variation due to differences in the levels of fertility of the bulls because pre-experimental fertility data for 12 of the bulls was lacking. A highly significant difference among bulls was obtained. Since the difference between breeds and the interaction of breeds and diluters were not significant, the interaction of bulls and diluters was used as the error term for calculating the *F* values for bulls and for diluters.

TABLE 4

Average fertility of semen diluted with heated homogenized milk and egg yolk–citrate containing antibiotics in Experiment 2

Breed	Diluters				No. bulls
	Milk		Egg yolk–citrate		
	(No. serv.)	*(% N. R.)*	*(No. serv.)*	*(% N. R.)*	
Guernsey	1,532	71.5	1,610	62.3	16
Holstein	2,646	71.7	2,612	65.3	14
Total	4,178		4,222		30
Services weighted equally		71.6		64.2	
Bulls weighted equally		71.0		63.5	

A separate analysis was made with only data from the 18 bulls for which pre-experimental fertility data on yolk–citrate–penicillin–streptomycin were available. The response in fertility of the individual bulls is shown in Table 5. A correlation was calculated between the differences in nonreturn rates on milk and yolk–citrate and the nonreturn rates for the bulls prior to the experiment when their semen was diluted routinely with yolk–citrate. A highly significant negative correlation of –0.65 was obtained. Bulls with a history of being lowest on yolk–citrate gave the largest increases in fertility when their semen was diluted with milk. It would have been very desirable to have more current pre-experimental fertility data for this comparison. Also, it is possible that the correlation value obtained was biased upwards. It was found that removal of four Guernsey bulls with rather meager fertility data on which to base classification into the high or low fertility group actually increased the correlation value from –0.65 to –0.79.

As in the first study, in the routine operation of the stud, semen was collected daily, except Sundays. In Experiment 2, however, none of the bulls selected was collected on Saturday to provide semen for use on Sunday and Monday. As a result, less than 5% of the inseminations were made on the second

TABLE 5

Comparison of milk and yolk–citrate diluted semen from bulls of different fertility levels in Experiment 2

Bull	12-mo. non-expt. yolk–citrate		2-mo. expt. yolk–citrate		2-mo. expt. heated milk		Experimental difference (Milk–yolk)
	(No. serv.)	*(% N. R.)*	*(No. serv.)*	*(% N. R.)*	*(No. serv.)*	*(% N. R.)*	
			Relatively high fertility bulls				
H43	3,488	72	267	71	264	68	−3
H45	2,722	71	264	66	270	72	+6
H54	915	71	173	76	136	78	+2
H58	889	70	310	71	290	77	+6
H38	2,126	66	201	65	232	72	+7
G43	2,256	68	107	67	131	65	−2
G61	1,641	67	85	74	118	77	+3
G47	2,296	66	207	67	161	70	+3
G48	231	65	85	67	92	75	+8
Total	16,564		1,699		1,694		
Bulls weighted equally		68.4		69.3		72.7	+3.4
			Relatively low fertility bulls				
H31	2,099	58	181	51	201	64	+13
G64	495	64	107	64	83	71	+7
G69	216	63	183	58	168	78	+20
G67	638	62	162	59	188	74	+15
G58	422	60	41	54	28	57	+3
G53	2,258	58	141	60	120	78	+18
G62	317	57	47	43	51	59	+16
G50	192	55	46	59	55	69	+10
G71	287	53	103	55	75	65	+10
Total	6,924		1,011		969		
Bulls weighted equally		58.9		55.9		68.3	+12.4

day after collection and insufficient data were available to make a valid comparison between diluters on the basis of the age of the semen at the time of use.

The results in Experiment 1 show that higher fertility was obtained when 1,000 units each of penicillin and streptomycin per milliliter were added to heated homogenized milk. The average improvement over milk without antibiotics of 5.9% was much greater for bulls of relatively low fertility (7.2%) than for bulls of relatively high fertility (2.3%). In the first fertility trials involving relatively fertile bulls Thacker and Almquist *(6)* reported that milk without antibiotics averaged 1.3% higher than yolk–citrate with antibiotics. A similar comparison in the present study involving only the six bulls of high fertility gave a difference of 1.9% in favor of milk without penicillin and streptomycin. Although milk without antibiotics provided satisfactory fertility results with fertile bulls in the present study and in previous work *(6)*, the routine addition of penicillin and streptomycin to heated milk diluter definitely is indicated.

Earlier favorable reports from this laboratory *(1, 6)* on the use of heated milk diluter concerned semen from bulls of relatively high fertility. The present

experiments strongly indicate that heated milk diluter containing penicillin and streptomycin provides a greater improvement in the nonreturn rates of relatively low fertility bulls than does egg yolk–citrate–penicillin–streptomycin. Bulls showing the lowest fertility rates with yolk–citrate diluter gave the largest differences in favor of heated milk diluter. Similar results were obtained by Dreher and Webb *(3)* in comparing heated homogenized milk and egg yolk–phosphate diluters, both containing penicillin and streptomycin. They found that bulls with the lowest nonreturn rates on yolk–phosphate diluter tended to show the greatest increases in fertility when their semen was diluted with milk.

SUMMARY

Fresh heated homogenized milk and egg yolk–citrate diluters for bull semen were compared in two split sample field experiments. In Experiment 1, semen from 12 Guernsey and Holstein bulls, six of relatively high fertility and six of relatively low fertility, was used to inseminate 6,670 first service cows. The 60- to 90-day nonreturn rate averaged 64.1% for yolk–citrate–penicillin–streptomycin diluter, 66.2% for heated homogenized milk diluter, and 72.1% for heated milk diluter containing penicillin and streptomycin. The difference of 8.0% in favor of heated milk with antibiotics over yolk–citrate with antibiotics was highly significant ($P < 0.01$). The increase of 5.9% obtained by the addition of penicillin and streptomycin to heated milk was significant ($P < 0.05$). No difference among diluters was obtained when comparing the nonreturn rates for semen used the first and the second days after collection.

Experiment 2 involved 240 semen samples from 30 Guernsey and Holstein bulls which were used to inseminate 8,400 first service cows. The average nonreturn rate was 63.5% for egg yolk–citrate–penicillin–streptomycin and 71.0% for heated milk–penicillin–streptomycin, or a highly significant increase of 7.5% in favor of milk. The difference between breeds was not significant.

On the basis of pre-experimental nonreturn data involving yolk–citrate with antibiotics, 12 bulls in Experiment 1 and 18 bulls in Experiment 2 were classified as either relatively high or low in fertility and used to compare heated milk and yolk–citrate diluters, both containing antibiotics. The difference in favor of milk was 4.2 and 3.4% for the high fertility bulls and 11.5 and 12.4% for the low fertility bulls in Experiments 1 and 2, respectively. There was a highly significant negative correlation between the nonreturn rates for the bulls in the pre-experimental period and the increase in nonreturn rates obtained with heated milk during the experimental period.

In conclusion, these results show that *(a)* the addition of penicillin and streptomycin improves the fertility of semen diluted with heated milk, *(b)* there is little difference between heated milk and yolk–citrate, both with antibiotics, for diluting semen from bulls of relatively high fertility, and *(c)* semen from relatively low fertility bulls generally shows higher fertility results when diluted with heated milk containing penicillin and streptomycin than when diluted with egg yolk–citrate containing antibiotics.

ACKNOWLEDGMENTS

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IDENTITY AND ORIGIN OF THE MALTY AROMA SUBSTANCE FROM MILK CULTURES OF *STREPTOCOCCUS LACTIS* VAR. *MALTIGENES*[1]

H. W. JACKSON AND M. E. MORGAN
Department of Animal Industries
Storrs Agricultural Experiment Station, Storrs, Connecticut

The development of the malty defect in dairy products appears to be the result of a peculiar bacterial fermentation about which little is known. The organism most commonly associated with this defect is *Streptococcus lactis* var. *maltigenes*. This organism appears to differ from *S. lactis* only in its ability to produce a malt-like aroma in milk *(3, 4)*.

Virtanen and Nikkilä *(12)* have described a "malt coccus" which they isolated from malty butter cultures. Although this organism differs from *S. lactis* var. *maltigenes* in its biochemical characteristics, it is noteworthy that these workers found it produces a considerable amount of acetaldehyde in milk and believe this compound to be responsible for the malty aroma. Simulation of the malty defect in normal butter cultures by addition of acetaldehyde seems to have led them to this conclusion.

Zuraw and Morgan *(13)* have found that acetaldehyde was liberated in milk cultures by *S. lactis* var. *maltigenes*, but also, and in most cases to a greater extent, by nonmalty strains of *S. lactis*. Their inability to simulate the malty defect in milk and normal lactic starters by addition of acetaldehyde further substantiates their claim that the characteristic aroma of cultures of *S. lactis* var. *maltigenes* is not related directly to the liberation of acetaldehyde.

The present investigation concerns the isolation, characterization, and origin of the volatile material responsible for the malt-like aroma of milk cultures of this organism.

EXPERIMENTAL PROCEDURE

Distillation of milk cultures. Eight cultures of *S. lactis* var. *maltigenes* and five cultures of *S. lactis* which had been isolated from different malty and nonmalty milk samples were employed in this study. One-liter quantities of fresh skimmilk were heated in flowing steam for 45 minutes, cooled, and inoculated with 2% of milk cultures of the test organisms which had been incubated for 24 hours at 25° C. The large cultures were incubated at the same temperature for 24 hours and immediately steam distilled. Thirty ml. of distillate was collected from each liter of culture and examined for odor. The distillates from the malty cultures had a pungent odor which was not entirely characteristic of the malty aroma. When the distillates were greatly diluted, the odor became more char-

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[1] This paper was prepared from data presented in a thesis by the senior author in partial fulfillment of requirements for the degree of Doctor of Philosophy at the University of Connecticut.

acteristic. Fresh milk to which small quantities of the distillate were added was judged as having a true malty aroma. Distillates obtained from nonmalty cultures of *S. lactis* did not impart a malty aroma to milk.

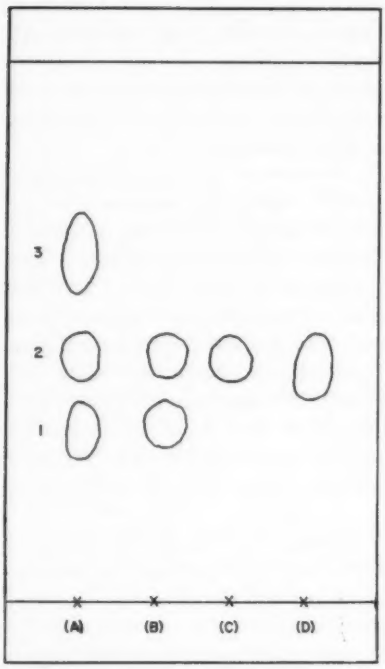
A distillate from a typically malty culture was redistilled from both acid and basic solutions. In each case the typical aroma material was distilled over, indicating that the aroma substance was of a neutral nature.

Preparation and chromatographic analysis of neutral carbonyl derivatives. Distillates from milk cultures of *S. lactis* and *S. lactis* var. *maltigenes* were examined for neutral carbonyl compounds by a procedure which was essentially that employed by Huelin *(5)*. Steam distillates of cultures were collected in a solution of potassium meta-bisulfite (final concentration 25 g. $K_2S_2O_5$ per 100 ml.) maintained at 0° C. Approximately 200 ml. of distillate was collected from 6-l. quantities of culture. The neutral carbonyl compounds were liberated from the bisulfite trapping agent by addition of an excess of sodium carbonate and redistilling. Approximately 100 ml. of this distillate was passed directly into 60 ml. of a 2,4-dinitrophenylhydrazine solution (0.1% in 1 *N* HCl) maintained at 0° C. The receiver was stoppered and the solution allowed to come to room temperature. The solution was then extracted with 50 ml. of a 1:1 mixture of benzene and heptane.

Extracts containing the 2,4-dinitrophenylhydrazones of the neutral carbonyl compounds were spotted in 0.02-ml. quantities on sheets of Whatman No. 1 paper so that the spots were 1.5 in. from the lower edge when the sheets were rolled and stapled in the form of cylinders. The developing solvent was prepared by shaking 25 ml. of methanol with 50 ml. of heptane (Eastman Practical Grade). The lower methanol-rich phase was placed in the bottom of a 6 by 12 in. cylindrical jar. The paper cylinder was placed in a 9-cm. petri dish cover resting on the bottom of the jar. The jar was sealed with a glass plate with silicone vacuum grease, and the paper was equilibrated over night. The heptane-rich phase was then added to the dish in which the paper cylinder was standing by means of a long-stemmed funnel inserted through a small hole in the jar cover. The hole was stoppered and the solvent allowed to travel up the paper for 2-3 hours. The paper was removed and the solvent allowed to evaporate. The 2,4-dinitrophenylhydrazones were readily discernible as yellow spots along the line of solvent flow. Dipping the chromatograms in 10% KOH or examination under ultraviolet light (3600A) tended to intensify the spots but was found to be unnecessary in this study.

A typical chromatogram of the 2,4-dinitrophenylhydrazones extracted from the distillates obtained from milk cultures of malty and nonmalty strains of *S. lactis* is shown in Figure 1. All malty strains tested gave three spots. Spot No. 3, the fastest moving spot, was always the largest and most intense. All nonmalty strains tested yielded but two spots in the same position as Spots No. 1 and 2 from the malty strains. An extract of derivative formed in a distillate from a heated milk control gave a single spot in the same position as Spot No. 2.

To determine whether Spot No. 3 was a derivative of an aldehyde or ketone the distillate from a malty culture was subjected to mild oxidation with freshly



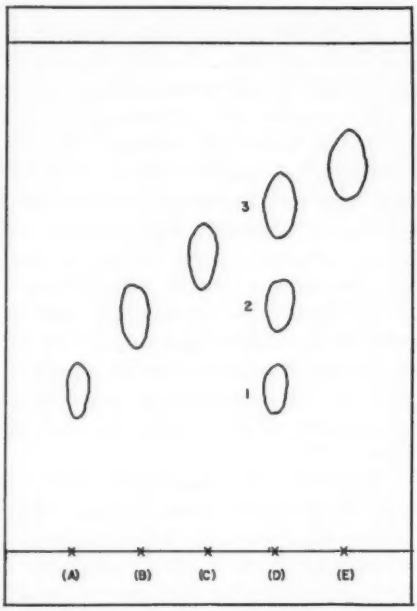


FIG. 1. Chromatogram of the 2,4-dinitrophenylhydrazones of the carbonyl compounds from distillates of *(A) S. lactis* var. *maltigenes* and *(B) S. lactis* milk cultures, *(C)* oxidized *(A)*, and *(D)* uninoculated culture milk.

FIG. 2. Chromatogram of 2,4-dinitrophenylhydrazones of *(A)* acetaldehyde, *(B)* acetone, *(C)* n-butanal, *(D)* carbonyl compounds distilled from *S. lactis* var. *maltigenes* milk culture and *(E)* n-hexanal.

precipitated silver oxide in an alkaline solution before redistilling into the 2,4-dinitrophenylhydrazine solution *(5)*. As shown in Figure 1, an extract of this mixture yielded but one spot, indicating that Spots No. 1 and 3 from the original distillate were derivatives of aldehydes and that Spot No. 2 was a derivative of a ketone.

The derivative mixture from a malty culture was chromatographed along with derivatives of several readily available neutral carbonyl compounds, as shown in Figure 2. Spots No. 1 and 2 of the unknown mixture ran parallel with the acetaldehyde and acetone derivatives, respectively. Spot No. 3 traveled to a position intermediate between the derivatives of n-butanal and n-hexanal, which suggested that the unknown spot was a derivative of a 5-carbon aldehyde.

N-pentanal and 3-methylbutanal were prepared from the corresponding alcohols by oxidation with sodium dichromate and sulfuric acid *(2)*. Fractions boiling within $\pm 3°$ C. of the boiling points of the respective pentanals were obtained

on redistillation of the above reaction products. 2-methylbutanal was obtained by simultaneous deamination and decarboxylation of DL-isoleucine (Merck) with ninhydrin at pH 2.5 and distilling *(11)*. The 2,4-dinitrophenylhydrazones of these aldehydes were prepared, extracted into benzene-heptane solvent, and evaporated to dryness. The residue from each preparation was dissolved in a minimum of solvent mixture and transferred to a column of anhydrous magnesium sulfate. The derivative was washed through the column with more solvent. This procedure removed material which usually remained at the origin when the derivatives were chromatographed *(5)*. The derivatives thus prepared were recrystallized from ethanol several times and chromatographed along with the unknown derivative mixture obtained from the malty milk culture distillate. As shown in Figure 3, Spot No. 3 of the unknown ran parallel with those of the three known derivatives. Since the 2,4-dinitrophenylhydrazones of carbonyl compounds having the same number of carbon atoms have the same Rf values when chromatographed with the methanol-heptane solvent *(5)* and since it has been shown that Spot No. 3 is an aldehyde derivative, it would appear that distillates from malty cultures contain a 5-carbon aldehyde or a mixture of pentanal isomers.

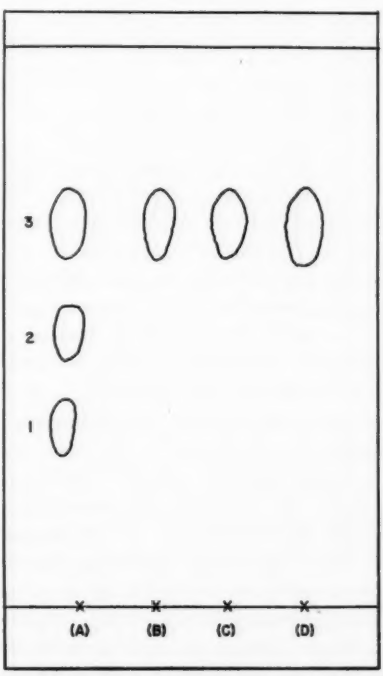


FIG. 3. Chromatogram of 2,4-dinitrophenylhydrazones of *(A)* carbonyl compounds distilled from *S. lactis* var. *maltigenes* milk culture, *(B)* n-pentanal, *(C)* 2-methylbutanal and *(D)* 3-methylbutanal.

Isolation and characterization of the pentanal derivative. To isolate sufficient material represented by Spot No. 3 for further characterization, the benzene-heptane extracts containing the unknown derivative mixture obtained from the distillates from approximately 24 l. of malty culture were pooled, evaporated to a convenient volume, and applied to numerous sheets of paper in an intense narrow band. Upon development of the papers, the derivatives were resolved into three well-defined bands. Band No. 3 was cut from the papers and eluted with methanol. The eluates were pooled and evaporated to dryness. The residue was dissolved in benzene-heptane solvent and passed through an anhydrous magnesium sulfate column. The solvent was evaporated and the derivative thus isolated was recrystallized from ethanol several times and dried. When chromatographed, the isolated derivative gave a single spot which ran parallel with the known derivatives of isomeric pentanals.

The melting points of the isolated derivative and the known derivatives taken on a micro-hot stage are shown in Table 1. The melting data for the unknown derivative corresponded most closely to those for the 3-methylbutanal derivative, although most of the unknown melted at a slightly lower temperature and over

TABLE 1

Melting points of the pentanal 2,4-dinitrophenylhydrazone isolated from milk cultures of S. lactis var. maltigenes and derivatives of three isomeric pentanals

Derivative	Melting points			
	Start	Most	Complete	Literature
	(C°)	(C°)	(C°)	(C°)
Unknown	117.0	120.5	123.0	——
n-pentanal	107.0	108.0	109.0	107.0 (2)
3-methylbutanal	121.0	122.0	123.5	123.0 (2)
2-methylbutanal	129.0	130.7	131.0	132.5-133.0 (1)

a greater range. Since further recrystallizations did not alter these values, the possibility of the aroma substance being a mixture of pentanals was considered. Speculation as to the possible precursors from which *S. lactis* var. *maltigenes* could produce branched chain pentanals in milk cultures led to the hypothesis that they are derived from leucine and isoleucine. If this were the case the unknown derivative would contain both the 3-methyl- and 2-methylbutanal 2,4-dinitrophenylhydrazones.

In order to test this hypothesis the infrared spectra of mineral oil-mulls of the unknown derivative and those of 3-methyl- and 2-methylbutanal were compared in a Perkin-Elmer Model 21 infrared spectrophotometer. No attempt was made to compensate for the mineral-oil absorption bands. Figure 4 shows the infrared spectrum of the unknown compared with spectra of authentic 3-methyl- and 2-methylbutanal 2,4-dinitrophenylhydrazones. Although the spectra of the latter two are similar, some distinct differences occur at several points. The 2-methyl compound has two absorption bands at 833 and 842 kaysers[2] whereas

[2] The term "kayser" (K) is used in this paper as a unit of wave number (formerly $cm.^{-1}$), as recommended by the Joint Commission for Spectroscopy *(6)*.

the 3-methyl compound has a single band at 833 K. The 2-methyl compound has weak bands at 1,015 and 1,097 K which are absent in the 3-methyl compound. Bands at 1,546 and 1,593 K also appear considerably stronger in the 2-methyl compound than in the 3-methyl compound. Other minor differences occur. Differences in the band near 725 K, however, are due to the relative amounts of mineral oil present. No significant differences in the spectra were found between 1,700 and 4,000 K. Examination of the spectrum of the unknown at the points of difference shows that it is clearly 3-methylbutanal 2,4-dinitrophenylhydrazone, with possibly a small amount of 2-methylbutanal 2,4-dinitrophenylhydrazone.

Organoleptic comparison of natural and synthetic malty aroma. Small quantities of dilute aqueous solutions of 3-methyl- and 2-methylbutanal were added to normal pasteurized milk until the odor of the respective aldehydes was just detectable by a judging panel. The panel's comparisons of the aldehyde-treated milks with a milk inoculated with *S. lactis* var. *maltigenes* indicated that the milk containing 3-methylbutanal possessed an aroma which was nearly identical with the natural malty aroma. Further attempts to modify the aroma produced by 3-methylbutanal by addition of various concentrations of 2-methylbutanal, acetaldehyde, and acetone resulted in no improvement. The concentration of 3-methylbutanal which gave the most typical aroma was 0.5 p.p.m.

Action of S. lactis *var.* maltigenes *cells on leucine and isoleucine.* In order to determine whether *S. lactis* var. *maltigenes* is able to convert leucine and isoleucine to 3-methyl- and 2-methylbutanal, respectively, the following experiment was performed. Cells of *S. lactis* var. *maltigenes* and *S. lactis* grown at 32° C. for 15 hours on trypticase soy agar (BBL) supplemented with 0.5% lactose and 0.5% agar were harvested in 0.67 *M* phosphate buffer at pH 7.0 *(10)*. The cells were washed twice with buffer. One-ml. quantities of packed *S. lactis* var. *maltigenes* cells were suspended in 25-ml. quantities of buffer with 10 mg. of L-leucine added, with 20 mg. of DL-isoleucine added and with no amino acid added. One ml. of packed *S. lactis* cells was suspended in 25 ml. of buffer containing 10 mg. of leucine. All suspensions were shaken several times during a 2-hour incubation at 37° C. The odor of 3-methyl- and 2-methylbutanal was readily detectable within 10 minutes in the *S. lactis* var. *maltigenes* suspensions containing leucine and isoleucine, respectively. The cells were removed by centrifugation and 15 ml. of 1% 2,4-dinitrophenylhydrazine in 1 *N* HCl was added to each of the supernatants. After 1 hour the carbonyl derivatives were extracted from the supernatants with benzene-heptane solvent. The extracts were concentrated to approximately 3 ml., and 0.02-0.04-ml. quantities were chromatographed by the procedure described previously. The resulting chromatogram shown in Figure 4 reveals that derivatives which ran parallel with the known derivatives of a 5-carbon aldehyde and acetaldehyde were extracted from the supernatants from both the suspension of *S. lactis* var. *maltigenes* incubated with leucine and that incubated with isoleucine. Only a derivative corresponding to that for acetaldehyde was extracted from suspensions of the malty organism incubated in the absence of amino acid. This also was the only derivative extracted from the suspension of *S. lactis* incubated with leucine.

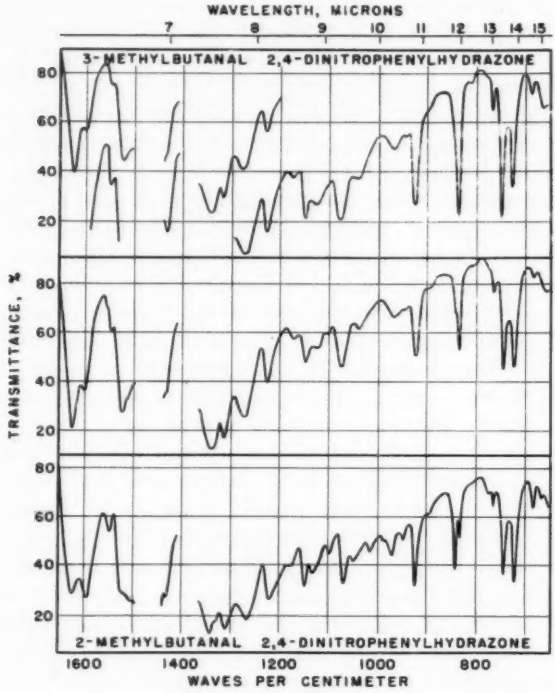


FIG. 4. Infrared spectra of mineral-oil mulls of 2,4-dinitrophenylhydrazones of 3-methylbutanal (upper curve), unknown pentanal (middle curve), and 2-methylbutanal (lower curve).

The remainder of the derivative mixture extracted from four replicate *S. lactis* var. *maltigenes*-leucine suspensions was applied in a continuous band to a single paper. The paper was developed and the derivative in the uppermost band was eluted and purified as described previously. This procedure yielded a few crystals which were sufficient for a micro-melting point determination. These melted at 122-123° C., which corresponded to the melting point of the authentic 3-methylbutanal derivative. No attempt was made to isolate the corresponding derivative from the derivative mixture obtained from the action of the organism on isoleucine because the concentration as estimated from the chromatogram precluded the possibility of obtaining sufficient crystalline material for a melting point determination from available extracts. However, since the uppermost spot on the chromatogram of this extract ran parallel with a known pentanal derivative it must be presumed that the organism is also able to convert isoleucine to 2-methylbutanal.

DISCUSSION

The infrared analysis of the pentanal 2,4-dinitrophenylhydrazones isolated from distillates of *S. lactis* var. *maltigenes* milk cultures indicated the presence

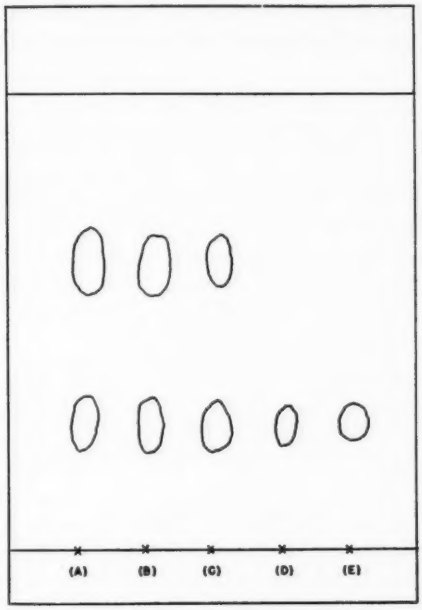


FIG. 5. Chromatogram of 2,4-dinitrophenylhydrazones of *(A)* acetaldehyde and 3-methylbutanal; and of the carbonyl compounds formed during incubation of *S. lactis* var. *maltigenes* cells with *(B)* L-leucine, *(C)* DL-isoleucine, and *(D)* without added amino acid and *(E) S. lactis* cells with leucine (approximately 1 ml. packed cells suspended in 25 ml. of 0.067 *M* phosphate buffer at pH 7.0 with equivalent of 10 mg. of the L-amino acids at 37° C. for 2 hr).

of principally 3-methylbutanal with only a small amount of 2-methylbutanal. Although the resting cell experiments indicated that the organism is capable of converting both leucine and isoleucine to the corresponding aldehydes, it appeared as though the organism was less active against isoleucine. This plus the fact that Morgan and Nelson *(8)* found that *S. lactis* cultures liberate about twice as much leucine as isoleucine from milk proteins during extended incubation may account for the preponderance of 3-methylbutanal in malty milk distillates. Since it has long been observed that the malty aroma becomes evident in milk inoculated with *S. lactis* var. *maltigenes* even before any appreciable increase in acidity occurs *(3)*, it is probable that the early precursor of the aroma substance is the free leucine of milk. Thereafter, an excess of leucine probably becomes available as a result of the proteolytic activity of the organisms *(8)*.

The biological conversion of leucine and isoleucine to the corresponding aldehydes, particularly in such a complex medium as milk, appears to be an unusual reaction. Since both *S. lactis (9)* and *S. lactis* var. *maltigenes (7)* have been shown to require both leucine and isoleucine for multiplication in an otherwise complete synthetic medium, it seems curious that the latter organism would make such improvident use of these amino acids.

The mechanism by which this organism converts these amino acids to pentanals is presently under investigation and will be the subject of a subsequent paper.

SUMMARY

Conclusive evidence is presented to show that the characteristic malty aroma of *S. lactis* var. *maltigenes* milk cultures is due principally to 3-methylbutanal. This fact was established by chromatographic resolution of the 2,4-dinitrophenylhydrazones of the neutral carbonyl compounds present in distillates of malty and nonmalty *S. lactis* milk cultures and the subsequent characterization of a pentanal derivative fraction isolated from the malty milk cultures.

Organoleptic tests revealed that as little as 0.5 p.p.m. of 3-methylbutanal added to milk simulates the characteristic aroma.

Although resting cell studies indicated that *S. lactis* var. *maltigenes* is able to convert both leucine and isoleucine to the corresponding pentanals, the isoleucine conversion appears to be of minor importance in milk cultures. Nonmalty *S. lactis* cells apparently are unable to convert leucine to 3-methylbutanal.

ACKNOWLEDGMENTS

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MICROBIOLOGICAL AND CHEMICAL STUDIES OF SWEET POTATO VINE SILAGE[1]

H. H. HALL,[2] J. L. ETCHELLS,[3] I. D. JONES,[4] AND W. M. LEWIS[5]
Agricultural Research Service, U. S. Department of Agriculture
AND
Departments of Horticulture and Animal Industry
North Carolina State College, Raleigh

This study was conducted as part of an investigation on the preservation of sweet potato vines by ensiling. Fresh vines, which yield an average of 10 to 20 tons per acre, are readily eaten by livestock, and it was believed they would be valuable as roughage for winter feeding if properly preserved. A review of the literature showed that Bennett and Gieger *(3)* ensiled sweet potato vines and mixtures of vines, phosphoric acid, and molasses in sealed fruit jars. These workers concluded that silage could not be made from vines alone but that good silage was made when phosphoric acid or molasses was added. In our investigation, data were sought from vines and mixtures of vines, tubers, and molasses by determining the progressive changes which occur in the microbial flora and chemical composition during the fermentation period. The work was conducted during 1941-1943 at the North Carolina State College with the cooperation of several departments (Agricultural Engineering, Animal Industry, and Horticulture) as well as the Agricultural Research Service of the U. S. Department of Agriculture. The results from feeding tests with sweet potato vines ensiled in silos will be reported separately by the Department of Animal Industry, North Carolina State College.

MATERIALS AND METHODS

Treatment of materials. Green, succulent vines and leaves (hereafter referred to as sweet potato vines) of the Puerto Rico variety of sweet potatoes were removed from the plants by hand or with a mechanical vine harvester, previously described by Hendrix *(9)*. Sweet potato roots were dug from the fields where the vines were harvested. Feed-grade molasses was used to increase the carbohydrate content of some silage. The silage mixtures were prepared by running the materials through a conventional silage cutter, which chopped the vines into approximately 1- to 1.5-in. lengths and the potatoes into irregular-shaped pieces about 0.25-0.75 in. in size.

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[1] Experimental work was conducted cooperatively between the Southern Utilization Research Branch and the North Carolina Agricultural Experiment Station. Paper No. 557 of the Journal Series of the North Carolina Agricultural Experiment Station.

[2] Fermentation Section, Northern Utilization Research Branch, Peoria, Ill.
[3] Fruit and Vegetable Section, Southern Utilization Research Branch, Raleigh, N. C.
[4] Horticulture Department, North Carolina State College, Raleigh.
[5] Present address: Biology Department, Southern Illinois University, Carbondale.

The composition of the barrel materials prepared each year is given in Table 1. Replicate 50-gal. wooden barrels each were filled with about 350 lb. of a mixture, and the material was packed firmly by treading. Thermocouples were placed in duplicate barrels of each treatment at about 10 to 12 in. from the top and bottom ends for the purpose of recording temperatures. After being

TABLE 1

Composition of materials ensiled in barrels for study of progressive microbial and chemical changes

Treatment	Composition
A	Sweet potato vines, 100%
B	Sweet potato vines, 85% and sweet potato tubers, 15%
C	Sweet potato vines, 83.5%, sweet potato tubers, 13.5%, and sugar cane molasses, 3%

Each barrel contained approximately 350 lb. of material. Barrels were filled during 3-year period as follows: 1941, on October 23; 1942, October 24; and 1943, October 12.

filled, the barrels were headed and stored under a roof shelter. Silages of the same composition made at the same time in 1-gal. glass jars with screw-type lids were used in this comparative study. However, the bacteriological and chemical studies reported here were conducted mainly on barreled silage. Samples of silage for analysis were obtained from duplicate barrels of each treatment by boring a 2-in. hole into the side of the barrel. A new hole was made at each sampling, after which it was closed with a stopper. One-pound samples were removed aseptically by a wire hook to sterile containers for bacteriological examination. Two-pound samples were similarly removed for chemical analysis. In 1941 no attempt was made to displace with gas the air in the void caused by removing the sample. However, in 1942 and 1943 the air was displaced with carbon dioxide to eliminate the effect of oxygen on the flora. Samples were obtained over a period of 69 days in 1941, 202 days in 1942, and 39 days in 1943. During 1941, 36 barrels of Treatment C silage were prepared for a statistical study of sampling methods to determine the effect on the flora, population of microorganisms, and chemical changes in barrels sampled repeatedly, as compared with previously unopened barrels. For this study the samples were taken at well-spaced distances from the barrels, which were opened repeatedly. Samples from previously unopened barrels were taken from the middle section. Silage made in glass jars was similarly studied. Only one sampling was made from each glass jar.

Bacteriological and chemical examination of silage. Media and other conditions for the cultivation and enumeration of the microorganisms have been described by Etchells and Jones *(8)*. Acid-forming and other mesophilic aerobic bacteria were grown on nutritive caseinate agar (Difco).[6] Coliform bacteria, primarily species of *Aerobacter*, were grown on brilliant green lactose-bile agar (Difco), and yeasts and molds were grown on acidified dextrose agar. The

[6] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

latter medium consisted of ordinary dextrose agar (Difco formula) to which 5 ml. of sterile 5% tartaric acid was added just prior to pouring the plates. Viable cell counts were made on these media from serial dilutions of silage washings (50 g. of sample shaken in 450 ml. of sterile water). Microscopic examination of the ensiled material was made by use of smears prepared from the silage washings by the method of Wang *(11)*, a modification of the Breed *(4)* technique. Microscopic counts of Gram-positive cells were made for large and small bacilli, large and small cocci, and yeasts.

Juice expressed from about 2 lb. of silage by a Carver hydraulic laboratory press was used for chemical analyses. pH was measured potentiometrically with a glass electrode. Sugars, as reducing sugar, were determined by the method of Shaffer and Somogyi *(10)*. Total acidity, expressed as lactic acid, was determined by titrating 10 g. of expressed juice (heated to a boil) with 0.1 *N* NaOH. Moisture was determined by drying in vacuo at 70° C.

RESULTS AND DISCUSSION

The initial composition of the material varied from year to year; yet the population trends of microorganisms and the chemical changes were the same within silage treatments. For this reason most of the data relative to progressive microbiological and chemical changes are presented graphically and represent average values for 3 years, except mold populations, which are for 2 years. The population trends for lactic acid-forming bacteria, *Aerobacter* sp., yeasts, and molds have been combined for all treatments for the 3 years and are shown in Figure 1.

Acid-forming bacteria. Lactic acid-forming bacteria were present in the ensiled material and comprised the majority of bacteria which grew on nutritive caseinate agar. This group predominated throughout the sampling periods. The average population of acid-forming bacteria varied initially from less than 10 thousand to 29 million per gram in the freshly barreled mixtures and increased rapidly during the first days of the fermentation. Maximum populations of about 150 to 600 million per gram were reached in about 5 days. After this there was a general decline in numbers throughout the sampling period. The majority of acid-forming bacteria were identified as *Lactobacillus plantarum*. This microorganism was likewise found by Allen *et al. (2)* to predominate in grass silage and to reach a population of 1 billion per gram after about 17 days. They observed the presence on the fresh grass of a mixed flora including lactobacilli, coliform bacteria, spore-forming bacteria, molds, and yeasts. Among these, *Bacillus subtilis* and *B. megaterium* also were found to increase greatly in numbers at the peak of the fermentation. Allen and coworkers also noted that thermophilic organisms identified as strains of *B. subtilis* increased in numbers with a rise in the temperature of the silage. Cunningham and Smith *(6)* observed strains of *L. plantarum* as well as *L. brevis* in silage made by the addition of mineral acids to plant materials. These workers reported that the flora consisted mainly of lactic acid–forming bacteria.

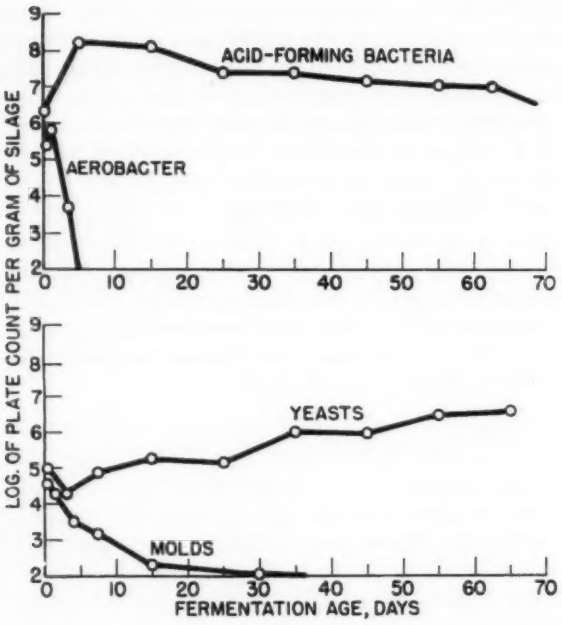

FIG. 1. Population trends of viable microorganisms in barreled silage.

Coliform bacteria. Variable numbers of coliform bacteria are present on the sweet potato vines, potatoes, and molasses. Figure 1 shows that the population of this group of organisms increased substantially during the first day and declined rapidly thereafter. The data suggest that the formation of lactic acid in the silage may be the limiting factor in the growth and survival of coliform bacteria. Etchells and coworkers *(7)* have shown that coliform bacteria do not survive during the fermentation of certain brined and salted vegetable material in which lactic acid is formed. These workers also demonstrated that coliforms were absent from fermentations that were acidified at the outset. Apparently these organisms contribute little to the fermentation of sweet potato vine silage.

Yeasts. Viable yeasts, comprising both budding and filamentous types, were present in all silage treatments throughout the sampling period. The initial counts ranged from 2 thousand to 1 million per gram and did not exceed 15 million per gram at any sampling. During 1942, the viable yeast population was exceedingly low and did not exceed 310 thousand per gram.

Yeasts characterized as the more oxidative, film-forming types were present to the extent of about 40 thousand cells per gram in the silage when first barreled. However, they were not observed on plating media until the 20th day of the fermentation. Since they were not present on platings from silage in barrels which had not been sampled previously, it is likely that they grew only in

barreled silage following admission of oxygen through repeated samplings. Thus, in silages that are poorly packed and exposed to air these yeasts might be expected to grow and utilize part of the acid and impair the keeping quality.

Molds. The initial mold counts of the barreled silage in 1942 and 1943 were 10 thousand and 79 thousand per gram, respectively. There was a gradual decline of the population beginning with the first day, and at 30 days mold colonies were almost completely absent from plated samples. There is no indication that molds contribute favorably to the fermentation of the silage.

It is of interest that in grass silage Allen and coworkers *(1)* noted a decrease in the amount of lactic acid toward the close of their experiments, which indicated to them that this acid was being decomposed, either by other groups of bacteria or by mold which was in evidence. The near absence of mold in our silage may be due, in part, to the physical properties of the ensiled material. Sweet potato vines have excellent packing qualities and do not provide much opportunity for the invasion of air.

Microscopic examination. Direct microscopic examination of washings from silage of each of the three treatments supplemented platings during the 1941 and 1942 seasons. Included were differentiations of types of microorganisms, measurement of cell sizes, and estimation of the maximum populations averaged for all treatments. These are given in Table 2. The size and appearance of these

TABLE 2

Microorganisms in sweet potato vine silage determined by microscopic examination

Microorganism	Size	Maximum population per g. $\times 10^6$ (average all treatments)	
	(microns)	*1941*	*1942*
Rods[a]			
large	0.9 × 2.7 to 4.0 0.9 × 6.3 to 15.0	1155	103
small	0.68 × 1.3 to 2.24	555	319
Cocci[a]			
large	1.4 to 1.8	63	31[c]
small	0.9 to 1.1	40	31
Yeasts			
fermentative[b]	2.3 to 3.6 × 3.6 to 4.5	72	6
oxidative	1.8 × 14.4 to 2.7 × 12.6	13	0

[a] Data are for Gram-positive organisms.
[b] Isolates identified were *Zygosaccharomyces* sp.
[c] Count of large and small cocci.

microorganisms remained uniform in the three silage treatments for these years. It is noted that the largest populations for each type of microorganism were obtained during 1941, a year during which the fermentation temperature exceeded all others. The differences are of such magnitude as to warrant consideration and, since they are greatest among the acid-forming organisms, they are treated in the forthcoming section on acidity. The combined average populations

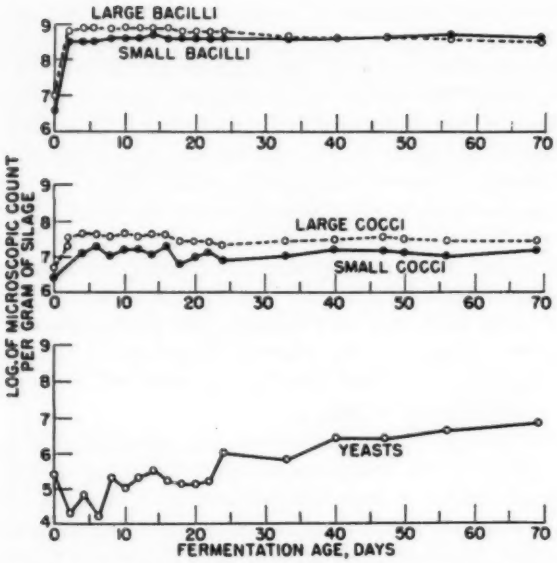

FIG. 2. Population trends of Gram-positive bacteria and of yeasts in barreled sweet potato vine silage.

of Gram-positive large and small bacilli, large and small cocci, and yeasts for 1941 are shown in Figure 2. The population trends are similar to those for viable acid-forming bacteria and yeasts. These results show that in the silage there is no reproduction of acid-forming bacteria after the initial active fermentation phase. These results also confirm the growth trend of yeasts in the silage. No attempt was made to determine the role of the different types of bacilli and cocci on the fermentation.

Chemical changes during fermentation of silage. Chemical analysis of the ensiled material shows some variation of its initial composition from year to year; however, examination shows that the chemical changes which took place during the fermentation followed the same course. For this reason progressive chemical changes, like the microbiological data, are presented graphically. The trends for pH, sugar, and acidity have each been combined for all treatments for the 3 years and are shown in Figure 3. The initial moisture contents of the vines was 78.5% for 1941, 83.7% for 1942, and 86.2% for 1943. The addition of potatoes and/or molasses lowered the initial moisture about 4.8%. The moisture content of the silage did not change appreciably during the fermentation.

pH. The initial pH of the chopped vines was 5.2 to 6.0 and showed only slight changes by the addition of potatoes or molasses to the vines. The onset of the fermentation was marked by an immediate and perceptible lowering of pH for about 15 days, after which there was no further change. The constancy of pH was additionally determined in 1942 when barrels of silage of each treatment

were examined after storage for 202 days. These results indicate that the most active stage of the acid fermentation was during about the first 15 days.

Sugars. Sugars in the press juices from the vines and potatoes occurred as reducing sugars; therefore, sugars added by molasses were determined as reducing sugars. The initial reducing-sugar content of juices from Treatments A and B for all years ranged from 1.92 to 3.58% and averaged 2.33%. The disappearance of sugar followed a similar trend for each silage treatment during the fermentation. For this reason, data from these treatments have been combined (Figure 3). Molasses increased the average sugar content of the juices from

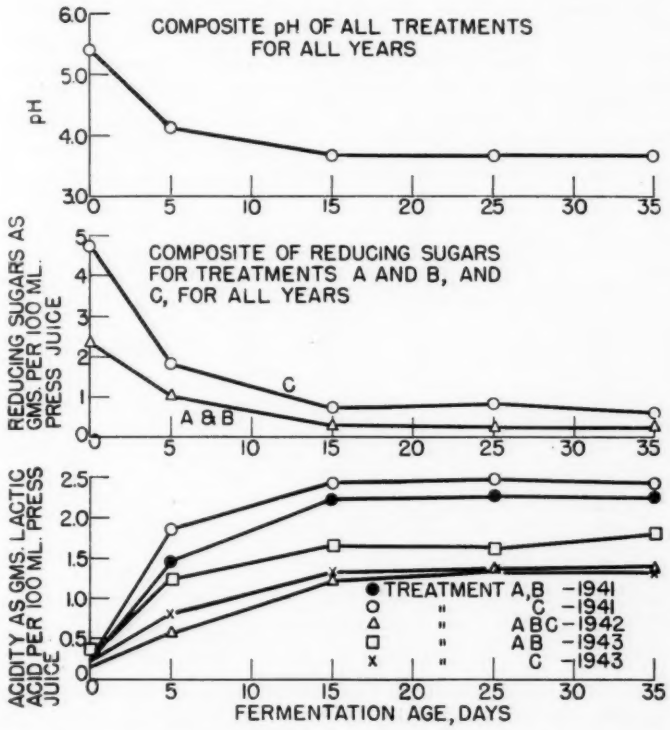
COMPOSITE pH OF ALL TREATMENTS FOR ALL YEARS
pH
COMPOSITE OF REDUCING SUGARS FOR TREATMENTS A AND B, AND C, FOR ALL YEARS
REDUCING SUGARS AS GMS. PER 100 ML. PRESS JUICE
C
A & B
ACIDITY AS GMS. LACTIC ACID PER 100 ML. PRESS JUICE
TREATMENT A,B -1941
C -1941
ABC -1942
AB -1943
C -1943
FERMENTATION AGE, DAYS

FIG. 3. Chemical changes occurring in barreled sweet potato vine silage.

silage of Treatment C to 4.75%. Data from the examination of the silage juices showed reduction of the sugar content beginning with the first day and continuing for about 15 days. There was no substantial further utilization of sugar beyond 35 days, as it was found that the residual sugar in Treatments A and B in 1942 was 0.15% and in Treatment C it was 0.25% in silage stored for 202 days.

Acidity. The data show that the formation of acid follows trends similar to those for pH, the disappearance of sugar, and also the population trend for

acid-forming bacteria. Although the initial acidity of the juice from all treatments of silage for the 3 years was relatively uniform, there were differences in the rate and amount of acid formed during the fermentation. All treatments attained maximum acidity in about 15 days, during the period when populations of viable bacteria were at a maximum. Temperature appears to influence the rate of acid formation and probably that of the activity of the acid-forming bacteria. Temperature data given in Table 3 show the average daily temperature

TABLE 3

Temperature of silage at sampling intervals

1941 season			1942 season			1943 season		
Date	Sampling interval	Silage temp.	Date	Sampling interval	Silage temp.	Date	Sampling interval	Silage temp.
	(days)	*(° F.)*		*(days)*	*(° F.)*		*(days)*	*(° F.)*
October			October			October		
23	0	76	24	0	59	12	0	—
25	2	75	25	1	—	13	1	67
27	4	66	26	2	—	14	2	68
29	6	71	27	3	59	15	3	70
31	8	64	28	4	53	16	4	71
			30	6	56	18	6	62
November						20	8	60
2	10	65	November			22	10	62
4	12	70	3	10	59	26	14	60
6	14	65	7	14	54			
8	16	64	13	20	53	November		
10	18	57	23	30	61	1	20	61
12	20	50				11	30	51
14	22	50				20	39	52
16	24	56	1943					
25	33	—	January					
December			8	77	38			
2	40	—	May					
9	47	51	13	202	—			
18	56	43						
31	69	47						
Av. temp. during first 20 days		65.7			56			58

of the silage at sampling times for the first 20 days of the fermentation to be 65.7° F. in 1941, 58° F. in 1943, and 56° F. in 1942. The rate of acid formation was likewise greatest in 1941, next in 1943, and lowest in 1942. There was no evidence of heating of the silage, and this may account for the prolonged period of high viable bacterial populations throughout the experiments. The expected relationship between the amount of acid formed and the initial sugar content of the silage mixtures was found only during 1941 and 1943 for the different silage treatments. During 1942, there was almost no effect on the total acids formed by the addition of molasses to the silage mixtures. This lack of difference may have been the effect of low fermentation temperatures. The disappearance of sugar in the 1942 silages, accompanied by low acid production, suggests that the low

temperature perhaps favored the fermentative activity of yeasts or other sugar–utilizing but nonacid–forming organisms. These combined data indicate, however, that within the limits of the conditions of the experiments, acid–type fermentations occurred in all treatments with the formation of lactic acid mainly, which resulted in the preservation of the ensiled materials. The odor, color, and physical characteristics of the silage from all of these treatments were typical of good quality silage.

Sweet potato vines ensiled with urea. During the 1943 season, a mixture of 99% sweet potato vines and 1.0% urea was ensiled in barrels to determine the effect of this nitrogen compound on the bacterial flora and chemical composition of the silage. The bacteriological data showed that urea did not exert a statistically significant difference with respect to time trends, or populations, except that this treatment supported the survival of *Aerobacter* sp. in a significantly higher number and for a longer period than did the three other treatments. Data showing populations and survival of *Aerobacter* sp. in these silages are given in Table 4. It will be seen that *Aerobacter* sp. survived for 8 days in silage composed of vines and urea, whereas they failed to survive more than 3 days

TABLE 4

The average numbers of coliform bacteria at different times on vines + urea and other treatments in 1943

Age of silage	Thousands per g. of silage	
	Vines + urea	Other treatments
(days)		
0	31.0	23.4
1	570.0	141.9
2	175.0	18.8
3	4.1	0.2
4	0.7	0.0
6	7.1	0.0
8	0.8	0.0
10-30	0.0	0.0

in the other treatments during this particular year. A comparison of the chemical data for silage composed of vines alone and that of vines and urea revealed similar trends for pH, acidity, and the disappearance of sugar. There are no important differences for these values. The silage containing urea had good odor, color, and texture throughout the sampling period.

The beneficial results of adding urea to silage have been reported by Cullison *(5)*. He reported that the prolonged fermentation period, which occurs when silage is made from sweet sorghum alone, was eliminated by the addition of 10 lb. of urea per ton of silage material. The resulting silage was superior in carotene content, palatability, and general feeding value to that made from sweet sorghum without urea.

Although the present study did not show a beneficial effect for urea on the fermentation of sweet potato vine silage, it could have the other values cited by Cullison. The present widespread use and recognized value of urea with

roughage feed for multistomach animals certainly suggest that it might be a useful additive to silage.

Analysis of data on experimental techniques. Several questions on experimental techniques were apparent at the outset of this study, and some of them were tested along with the major objectives. It was desired to know the effect of different sampling methods on the flora, population of microorganisms, and the chemical changes in barrels sampled repeatedly, as compared with previously unopened barrels. There was also the question of the effect of the type of container on the flora, particularly glass containers, which were used in other investigations (Bennett and Gieger, *3*). Some of these data were analyzed statistically to determine the more precise effect of these variables on the microbial populations and growth trends.

Effect of different sampling methods. Two methods were studied in 1941 in following the course of the fermentation: *(a)* taking samples from the same barrels on successive dates and *(b)* taking samples from new barrels opened each time. The bacteriological results for the two methods did not differ significantly with respect to time trends, or mean or peak numbers of acid-forming bacteria, but yeast counts were decidedly lower when new barrels were opened each time. These differences are shown graphically in Figure 4. It was assumed

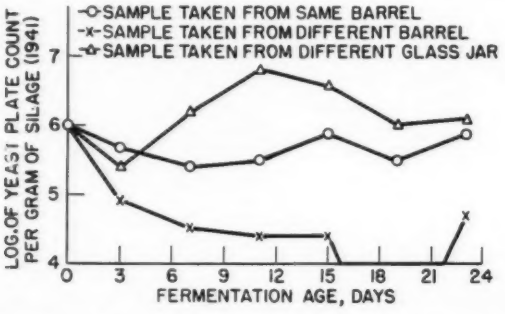

FIG. 4. Population trends of viable yeasts in silage taken each time from the same barrel versus a different barrel and from a different glass jar.

that the fundamental difference between the two methods was one of aeration, and that this was a more important factor in yeast development than in acid-forming bacteria.

Effect of type of containers. Wooden barrels were compared with glass jars as suitable containers for such experiments when a new container was opened at each sampling date. The glass jars gave significantly higher yeast and mold counts as well as more irregular time trends than the barrels. These differences also are shown in Figure 4. The reason for these variations is not known but they may be due, in part, to temperature. The temperature of the silage in the jars was more irregular than that of the barrel silage in that it more nearly

corresponded to that of the atmosphere. The disappearance of sugar from silage in glass jars was not determined, but the pH drop and formation of acid was less than that of the barreled silage. Nevertheless, the silage in glass jars had good color, odor, and texture and appeared like that in the barrels.

Sources of experimental error. Difficulties of handling nonnormally distributed data such as result from studies with this type material (leaves, stems, and tubers) usually result in the use of approximation techniques. Nevertheless, the results are accurate enough to permit sound conclusions about the relative importance of different sources of experimental error. The relative magnitude of errors from the different sources was approximately the same for acid–forming bacteria and yeasts. Expressed as coefficients of variation they are:

Plating and counting	6%
Samples within a barrel	50%
Barrels with the same treatment	15%

In determining the most efficient technique to use, three considerations must be taken into account: *(a)* the precision desired, *(b)* the expense and effort involved in using more barrels compared to taking more samples per barrel, and *(c)* the limiting number of barrels and samples which can be handled. Table 5 shows

TABLE 5

Least significant difference ($P = 0.05$) in acid-forming bacteria expected from varying the number of barrels and samples per treatment (expressed as percentage of the higher treatment mean)

Samples per barrel	Barrels per treatment			
	2	4	6	8
1	90	54	42	36
2	83	42	32	28
4	69	33	25	21
8	58	27	20	17
16	50	22	17	13
32	44	19	15	12

the relative accuracy that could be expected by varying the number of barrels and samples. For example, assume a new experiment was planned in which each treatment was placed in four barrels and two samples per barrel drawn. If Treatment A had 100 million acid–forming bacteria per gram, Treatment B would have to be down to 100–42, or 58, million per gram to be significantly different.

SUMMARY AND CONCLUSIONS

Sweet potato vines and combinations of vines, tubers, and molasses were ensiled during consecutive years in glass jars and wooden barrels. There resulted under each condition an acid–type fermentation with the production of silage having good odor, color, and texture. During the most active phase of the fermentation, the population of acid–forming bacteria, which was mainly *L. plantarum*, increased rapidly from 150 to 600 million per gram of silage in

about 5 days. Although the population of acid-forming bacteria varied from year to year, their numbers were not greatly influenced by the addition of sweet potato tubers or molasses to the vines.

Chemical changes during the fermentation were evidenced by a drop in pH, a decrease in the sugar content, and the formation of lactic acid. The addition of tubers and molasses to vines increased the amount of acid formed, over vines alone, except one year when low temperatures prevailed throughout the active fermentation phase.

Analysis of data on techniques shows different sources of experimental error to be 6% for plating and sampling, 50% for samples taken repeatedly from the same barrel, and 15% for samples taken each time from different barrels.

ACKNOWLEDGMENTS

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THE EFFECT OF VARIOUS FACTORS ON THE VITAMIN D CONTENT OF SEVERAL COMMON FORAGES[1]

H. A. KEENER

Department of Dairy Husbandry
New Hampshire Agricultural Experiment Station, Durham

There is adequate evidence in the literature to prove the need for vitamin D by dairy cattle. Although direct solar irradiation can provide an animal with the antirachitic factor, the amount of vitamin D in the ration is of primary importance in determining the adequacy of this factor, particularly during the winter months. Because forage crops are the principal natural source of vitamin D in the ration of the cow, the need for vitamin D supplementation depends on the amount provided by the forage.

It has been believed generally that green forage plants are devoid of vitamin D and that this vitamin is formed in the plant upon exposure to sunlight after mowing. Unfortunately, the situation is not nearly as simple as this. As shown by Wallis *et al. (10)*, the vitamin D content of different hays varies widely and cannot be predicted accurately on the basis of present knowledge. Many other reports in the literature as reviewed by Thomas and Moore *(8)* support this observation.

Newlander *(7)* reported that in 1946 artificially dried hay cut June 27 and July 1 contained about twice as much vitamin D as comparable sun-cured and barn-cured hays. The next year sun-cured and barn-cured hays cut on July 14, 16, and 24 contained three to four times as much vitamin D as comparable artificially dried hays and about three to four times as much vitamin D as the sun-cured and the barn-cured hays made in 1946. There was little difference in the vitamin D content of sun-cured and barn-cured hays made from the same field at the same time. Moore *et al. (6)* reported 2 years work on alfalfa harvested simultaneously as field-cured hay, barn-cured hay, and wilted silage. The first year the field-cured hay contained twice as much vitamin D as barn-cured hay or wilted silage on an air-dry basis. The second year wilted silage was equal to field-cured hay in vitamin D content and about 50% higher than barn-cured hay. Thomas and Moore *(8)* found that the antirachitic activity of an alfalfa crop when cut increased with the stage of maturity and the amount of dead material present. The percentage of dead material increased with maturity.

The work reported here was carried out as a result of the inconclusive results obtained from a study to determine the value of supplemental vitamin D for lactating cows. The roughages fed were found to vary so much in vitamin D content that it seemed necessary to first determine the conditions under which a shortage of vitamin D might occur in the ration of dairy cattle before carrying

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TABLE 1

Vitamin D content of miscellaneous forages and conditions of harvesting and storage

Type of roughage	Origin	Date cut	Time in field after mowing	Method of curing or storage	Date sampled	Vit. D content as fed
						(IU/lb)
First cutting mixed hay	Graham farm	June 15, 1946	24 hr.	Mow-cured	Dec. 11, 1946	170
First cutting mixed hay	"	July 1, 1946	3-4 days	Field-cured	Composite-winter 1946-47	830
Second cutting mixed hay	"	Sept., 1946	48 hr.	Mow-cured	Dec. 11, 1946	1724
Second cutting mixed hay	"	Aug. 5, 1947	24 hr.	Mow-cured	Dec. 23, 1947	590
Soybean hay	"	Aug. 10, 1947	48 hr.	Mow-cured	Dec. 23, 1947	322
Grass silage mixed	"	June 10-20, 1946	1-3 hr.	Wilted, no preservative	Composite-winter 1946-47	83 (200) [a]
Corn silage	"	Sept. 1-10, 1946	none	Field-chopped	Composite-winter 1946-47	63 (193) [a]
Corn silage	Hampshire Hills farm	Sept. 1-10, 1947	none	Field-chopped	April 16, 1948	62 (249) [a]
Corn silage	Rice Brothers	Sept. 1, 1947	none	Field-chopped	April 16, 1948	85 (282) [a]
Red and ladino clover hay	N. H. Agr. Expt. Sta.	Sept. 9, 1947	5½ hr.	Mow-cured	March 19, 1948	195
Timothy hay 10% heads showing	"	June 21, 1947	51 hr. (good weather)	Field-cured	March 19, 1948	259
Timothy hay early bloom stage	"	July 5, 1947	55 hr. (mostly good weather)	Field-cured	March 19, 1948	295
Timothy hay seed stage	"	July 25, 1947	7 hr. (good weather)	Field-cured	March 19, 1948	358

[a] Calculated to hay equivalent basis (90% dry matter).

out further work of this type. This work was an attempt to determine how certain factors affect the vitamin D content of forage.

EXPERIMENTAL PROCEDURE

The hay samples listed in Table 1 were obtained from various sources in order to get a general idea of the vitamin D content of several types of roughage. The hay samples were ground for assay without further drying. The silage samples were oven dried before grinding.

In the work designed to study the effect of curing time and the method and length of storage on vitamin D content as reported in Table 2, samples of fresh, green forage were obtained by picking out individual species immediately behind the mowing machine. The wilted silage, mow-cured hay, and the field-cured hay

TABLE 2

Vitamin D content of different species of forage as affected by curing time and by method and length of storage (dry basis)[a] *1948-49*

Method and stage of storage	Alsike clover 1st cutting	Ladino clover 1st cutting	Ladino[b] clover 2nd cutting	Red clover 1st cutting	Timothy 1st cutting
	(IU/lb)	*(IU/lb)*	*(IU/lb)*	*(IU/lb)*	*(IU/lb)*
Green forage					
When cut—July 7	23	23	204	27	23
Wilted silage					
When stored—July 8	168	91		36	36
When fed—Jan. 12	150	209		181	77
Mow-cured hay					
When stored—July 9	195		188	70	73
When dry—Aug. 10	190		234	127	113
When fed—Jan. 12	227		227	113	73
Field-cured hay					
When stored—July 10	195		331	75	104
When fed—Jan. 12	145		386	113	82

[a] All values based on drying in convection oven at 70° C.
[b] Mowed July 8 in another field because of scarcity of ladino clover in field being harvested.

samples were obtained by picking out samples of the individual species from the windrow just before the forage was picked up by the forage harvester in the respective operations. Samples were collected in multiwalled paper bags to prevent further exposure to sunlight. Each sample of the forage being ensiled was divided into two parts, one for immediate drying for assay, the other for storage in the silo. The hay samples were divided in the same way except that an extra sample of each species was prepared from the hay to be mow-cured. All samples intended for storage were placed in porous cotton bags, tied, buried in the appropriate silo or mow, and covered with the forage from which the samples were taken. When the mow-cured hay had become dry, the extra sample of each species was removed and prepared for assay. When the silage samples were uncovered, all remaining samples were removed from storage, dried in a convection oven at 70° C., and prepared for assay.

As shown in Table 4, additional samples were obtained in three different years to study factors affecting the vitamin D content of forages when freshly cut. These samples were collected between 1:30 and 3:00 P.M. on bright, sunny days. The forage was harvested with shears and placed immediately in multi-walled paper bags. Samples were dried in the convection oven over night and prepared for assay by grinding in a Wiley mill fitted with a 1-mm. screen. Portions of these samples were subjected to ultraviolet irradiation and again assayed for vitamin D as a means of estimating possible differences in their ergosterol (provitamin D) content.

The irradiation studies were carried out by placing 70 g. of the dry ground forage in a fused quartz cylinder which was rotated on its horizontal axis at a standard distance from the quartz mercury vapor lamp of an R.U.V. ultraviolet apparatus. The quartz cylinder, which was 5½ in. long and 3½ in. in diameter, was rotated at the rate of 5.9 times per minute by an electric motor. The forage was thoroughly agitated by the resulting tumbling motion. The irradiation procedure was timed with a stopwatch. A photograph of the rotating quartz cylinder is shown in Figure 1.

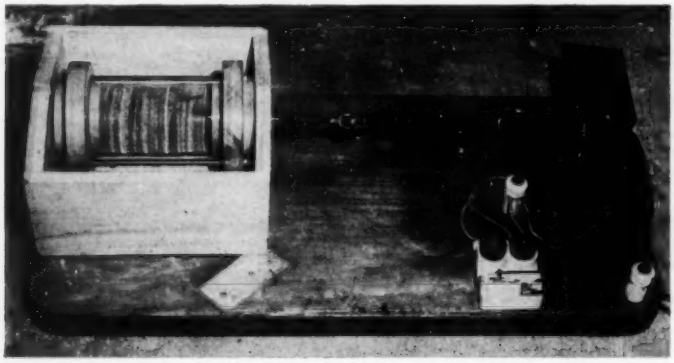

FIG. 1. Photograph of rotating quartz cylinder used in irradiating ground forages.

All ground samples were placed in air-tight glass jars and stored in a freezer until assayed.

The vitamin D content of all samples was determined by the rat assay method essentially as set forth in the U. S. Pharmacopoeia XII *(9)* and by a curve of response essentially as outlined by Coward *(1)*.

RESULTS AND DISCUSSION

The results given in Table 1 show a wide range in vitamin D content. The hays from the Graham farm, which were used in the vitamin D experiment with lactating cows, varied from 170 units per pound in an early cut mow-cured hay to 1,724 units in a second cutting mow-cured hay. These data indicated a possible tendency for second crop forages to be somewhat higher in vitamin D than first cuttings.

The silages were all essentially the same with respect to vitamin D content. These results indicated that on a dry matter basis corn silage was as good a source of vitamin D as some hays and some grass silages.

The three timothy hays at the bottom of Table 1 did not show much increase in vitamin D content with increasing maturity, but the short curing time in the seed stage sample may have held down the vitamin D content to some extent.

The data in Table 2 show the vitamin D content of the various species as affected by curing time and by method and length of storage. All of the first cutting samples were practically devoid of vitamin D when cut, whereas the lush second cutting ladino clover from a different field contained an appreciable amount of vitamin D when cut.

Upon exposure to sunshine the alsike clover in this trial almost reached a peak in vitamin D content after 1 day in the field. The red clover appeared to reach a plateau after 2 days while the timothy showed increasing values for 3 days, at which time all samples were stored as field-cured hay.

The second cutting ladino clover remained essentially unchanged for 1 day but showed a significant increase for the second day. The final value was about 70% higher than the field-cured hay made from first cutting alsike clover and three to four times higher than the first-cutting red clover and timothy hays. Possible reasons for the higher values for the second cutting ladino clover will be discussed later.

It is of interest to note that three of the four samples of wilted silage showed a marked increase in vitamin D between time of ensiling and when fed 6 months later. The silages when fed contained essentially the same amount of vitamin D on a dry matter basis as the field cured hay from the same species. This apparent increase in the vitamin D content of silage during storage appears worthy of further investigation.

In an attempt to determine the period of time which should be selected as standard for the irradiation studies, the data shown in Table 3 were collected.

TABLE 3

Effect of irradiation time on vitamin D content of various forages (dry basis)[a]

Type of forage	Date sampled	Length of irradiation period			
		None	5 min.	10 min.	20 min.
		(IU/lb)	*(IU/lb)*	*(IU/lb)*	*(IU/lb)*
Timothy					
1st cut hay	9-15-48	499	1,225	907	1,043
Field-cured hay	1-12-49	82	138	431	265
Red clover					
Wilted silage	1-12-49	181	86	567	209
1st cut hay	6-15-49	172	209	141	227
Ladino clover					
1st cut hay	9-15-48	816	1,923	1,678	
2nd cut hay	9-16-49	263	680	671	

[a] All values based on drying in convection oven at 70° C.

This work was carried out to establish a procedure for estimating the provitamin D content of the plant material. In some cases the maximum vitamin D activity was obtained in 5 minutes, in others in 10 minutes, and in still others in 20 minutes. Because the 10-minute interval gave the highest average value, this was selected arbitrarily as standard. It appeared that after a maximum value was obtained, further irradiation often resulted in loss of activity. In the case of the one sample of silage, it appeared that a loss occurred before any appreciable increase took place.

The effects of time of cutting, stage of maturity, and species on the vitamin D and provitamin D content of forage plants when cut are shown in Tables 4 and 5. In 1948 all species were very low in vitamin D at the first cutting in June, and the levels were increased by irradiation only to the lowest levels found in the

TABLE 4

Vitamin D content and potential vitamin D content of several forages as affected by various factors (dry basis)[a]

Cutting and date	Bromegrass		Ladino clover		Red clover		Timothy	
	As cut	Irradiated	As cut	Irradiated	As cut	Irradiated	As cut	Irradiated
	(IU/lb)	*(IU/lb)*	*(IU/lb)*	*(IU/lb)*	*(IU/lb)*	*(IU/lb)*	*(IU/lb)*	*(IU/lb)*
			Pine Tree field 1948					
First cutting								
June 25			27	227	14	159	18	41
August 2			308		340		259	
Sept. 15			816	1,678	635	2,540	499	907
Second cutting								
August 2			200		59	240	195	454
Sept. 15			431		445		372	
Third cutting								
Sept. 15			177	454	227	150	245	544
			Hoitt field 1949					
First cutting								
June 15			36	254	172	141	159	168
Second cutting								
Sept. 16			263	671	154	417		
			Usher and Moore fields 1951					
First cutting								
June 13	50	195	50	204	50	63	45	63
Second cutting								
Sept. 4	113	417	77	245	50	136	73	422

[a] All values based on drying in convection oven at 70° C.

hays in Table 1. In 1949 the corresponding first cutting samples contained about the same amount of vitamin D when cut as the 1948 samples after irradiation, but except for the ladino clover little change resulted from irradiation. In 1951 the situation was essentially the same as in 1948 except that red clover as well as timothy remained low.

TABLE 5

Vitamin D content of first cutting and comparable second cutting samples after irradiation (dry basis)[a]

Species	Year	First cutting	Second cutting
		(IU/lb)	*(IU/lb)*
Bromegrass	1951	195	417
Ladino clover	1948	227	454[b]
	1949	254	671
	1951	204	245
	Av.	228	457
Red clover	1948	159	240
	1949	141	417
	1951	63	136
	Av.	121	264
Timothy	1948	41	454
	1951	63	422
	Av.	52	438
All species	Av.	150	384

[a] All values based on drying in convection oven at 70° C.
[b] Third cutting same, no second available.

It is interesting to note that the June cuttings of ladino clover showed a marked increase in vitamin D upon irradiation in each of the 3 years. Timothy, however, showed only very modest increases in vitamin D for the 3 years. Red clover showed a sharp increase in 1948 and practically no increase for the other 2 years. The pattern is quite variable, although there is a suggestion of a species difference. In every case during the three seasons the vitamin D content of the first cutting samples harvested in June remained relatively low even after irradiation.

Second cutting samples taken at comparable stages of maturity as the first cutting samples harvested in June contained on the average 2.5 times as much vitamin D when cut and about 2.5 times as much vitamin D after irradiation as the comparable first cutting samples. The difference between first and second cutting irradiated samples as reported in Table 5 was found to be highly significant. There was also a significant difference in irradiated values between species in the first cutting, but not in the second. Both red clover and timothy were inferior to the other species in the first cutting and red clover also tended to be low in the second. There did not appear to be any consistent difference between years as far as provitamin D was concerned.

The forages harvested at a more mature stage were much higher in vitamin D when cut than the corresponding immature species. When irradiated, these mature forages were found to increase markedly in vitamin D content. It would appear that the higher provitamin D and vitamin D values of forages were associated with increasing maturity of the plant. The amount of dead plant tissue may be of only secondary importance.

The results of this study indicate that immature first cutting forage is likely to be a relatively poor source of antirachitic potency even when cured in the field. Second cutting forage of equivalent stage of maturity is on the average a potentially better source of this activity. The higher vitamin D content of more mature forage is of considerable scientific interest but of little apparent practical importance because of the lowered feeding value in other respects.

The irradiation studies might be interpreted to indicate that the difference in the vitamin D activity between the first and second cutting forages was due to a difference in ergosterol content and the extent of ultraviolet irradiation. It is difficult, however, to explain on the basis of these simple relationships why the vitamin D content of silage made from three species of plants as reported in Table 2 increased an average of 2.8 times during storage to reach the approximate level of the corresponding field cured hays, whereas the fourth species, alsike clover, which had already reached the approximate level for this crop as field cured hay, did not change appreciably.

One possible explanation for this situation is that vitamin D is present normally in the growing plant, but its biological effect is counteracted in varying degrees by an antivitamin D. This explanation is suggested by the results of work published since 1948. Ewer and Bartrum *(3)* and Ewer *(2)* reported that some factor in artificially dried green oats caused the development of rickets in sheep. Grant *(4)* found a similar activity in the chloroform extracts of dried young oats, Italian rye grass, and even young green permanent pasture. An extract of the dead leaves had the opposite effect. Weits *(11)* reported that when vitamin D was fed to rats along with an extract of hay, there was a 20 to 50% loss of the vitamin D activity. This was said to offer an explanation for the apparent absence of vitamin D in fresh green forage at certain times and not at others. Recent work by Grant *(5)* indicates that the antivitamin D found in plant material is carotene. Vitamin A is reported to have a similar effect.

In light of the above, the excess of the vitamin D activity over antivitamin D activity would be measured in terms of antirachitic potency. It would seem that instead of functioning only in the formation of vitamin D, ultraviolet light also might inactivate the antivitamin. If this explanation is correct, it would appear that things other than ultraviolet light also might inactivate the antivitamin D. The action of the fermentation process in the silo might be an example of this. While this explanation is given as theory and not necessarily fact, there is some evidence to support it. Further work is needed to clear up this question.

SUMMARY

An experiment was carried out to determine the effect of species, season, stage of maturity, and method of storage on the vitamin D content of forage.

It was found that the vitamin D activity of fresh green plants was variable but of a low order. First cutting red clover and first cutting timothy were potentially poorer sources of vitamin D than the other species studied. First cutting forages of all species were potentially much poorer sources of vitamin D

than subsequent crops cut at similar stages of maturity. Forages cut at later stages of maturity were higher in vitamin D than the same forages cut at early stages of maturity.

First cutting mow-cured hay, field-cured hay, and grass silage made from the same field at the same time all had essentially the same vitamin D activity on the oven dry basis when fed. A particularly interesting observation was the apparent increase in antirachitic activity in forage during storage in the silo. The destruction of an antivitamin D factor may offer an explanation for this phenomenon. Further work on this problem is needed.

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FACTORS RESPONSIBLE FOR THE DEVELOPMENT OF A HAY-LIKE FLAVOR IN VITAMIN A FORTIFIED LOW-FAT MILK

K. G. WECKEL AND ETZER CHICOYE
Department of Dairy and Food Industries, University of Wisconsin, Madison

Modified low-fat milk has become an important bottled milk product in many cities *(3)*. It consists of skimmilk, or skimmilk modified by the addition of either or both 0.25-0.75% fat and 0.5-2.0% milk solids-not-fat *(6)*. The vitamin content is usually increased to levels of at least 2,000 I.U. vitamin A and 400 I.U. vitamin D per quart by use of processed vitamin concentrates. Vitamin A in the form of a natural derivative from marine oil, or as a vitamin A palmitate or acetate, and vitamin D as a natural derivative from marine oil, or as D_2 or D_3, are used *(12)*.

A distinct abnormal flavor occasionally develops in such modified low-fat milk and is present invariably only when fortified with vitamin A *(11)*. The occurrence of the flavor is irregular and often spasmodic. A study was undertaken to ascertain the factors responsible for its development.

The flavor defect hereinafter designated as a hay-like flavor is perhaps best described by that term. Other terms that may help describe it are alfalfa, carrot-like, and perfume. The flavor can be readily differentiated from oxidized flavor associated with the fat or the activated flavor due to the action of radiation on certain protein fractions of milk *(4, 9, 10)*. The flavor, in commercial practice, seems to develop more frequently only after the pasteurized low-fat milk is 48-72 hours old. Often it becomes apparent in the milk product held over as "route returns" and when stored in a refrigerator for several days. The flavor may develop in the milk product of one dairy in a community, but not in another using seemingly comparable conditions of operations and the same vitamin concentrate product. Frequently, the flavor defect may appear in one portion of the output of a given dairy, but not in another.

PROCEDURE

A survey was made of the methods of processing low-fat milk in a number of plants throughout the country where the flavor defect was known to occur. The survey data aided in planning the experimental study. The development of the hay-like flavor was studied by experimental variation of three conditions: *(a)* the composition of the modified low-fat milk; *(b)* the methods used in its processing, and *(c)* the treatment subsequent to its processing.

Freshly separated skimmilk was selected as the "medium" for the study after preliminary trials indicated that the defect developed as readily in it as when added fat, or solids-not-fat, were present. Commercial vitamin A and D concentrates, homogenized in milk constituents, were used to fortify the milk. Separate lots of skimmilk were pasteurized in an experimental four-compartment

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vat pasteurizer, using various temperature and time treatments. The skimmilk was processed in batch quantities of 30 lb., portions of which were subsequently packaged in various containers and subjected to various conditions of storage.

Flavor tests of the skimmilk were made every 24 hours by a staff taste panel, using rank-order procedure *(2)*.

RESULTS

Several potential factors were eliminated in a preliminary way by a series of tests as less significant causes of the hay-like flavor. Among the factors thus eliminated were: differences in various types and forms of vitamin A concentrate; differences in amounts of vitamin A added (up to 4,000 units per quart); the point at which the vitamin concentrate was added during the processing of the milk, i.e. at intervals before, during, or after pasteurization; the age of the vitamin concentrate; the presence of added psychrophilic organisms in the pasteurized skimmilk; aeration with oxygen during processing. None of the above factors alone appeared to be especially important in causing development of the flavor, although collectively they may be significant.

Several factors did have a definite effect upon the development of the hay-like flavor. Among these were:

1. *Exposure of skimmilk to sunlight.* Exposure of milk to sunlight usually results in the development of either oxidized or activated flavors. Brief exposure of whole milk often results in the development of an oxidized flavor during subsequent storage, whereas longer exposure periods result in the development of an activated flavor usually apparent immediately. It was observed in these experiments that when either vitamin A fortified or nonfortified skimmilk in clear glass bottles was exposed to sunlight for intervals of 2 to 3 minutes and then subsequently stored in the dark, a change of flavor became detectable in both. During the storage interval a distinct hay-like flavor developed in the milk fortified with the vitamin A, but not in the milk without added vitamin A. The usual interval for the appearance of the oxidized flavor was about 24-48 hours and for the hay-like flavor, 48-72 hours. The results of such a series of tests are presented in Table 1.

2. *Exposure of skimmilk to fluorescent light.* Exposure of skimmilk, with or without added vitamin A, to fluorescent light also resulted in the development of undesirable flavors. When the skimmilk in clear glass quart bottles was exposed to the radiation from a 40-w. soft white fluorescent lamp at a distance of 12 in., a slight change in flavor became apparent within 12-24 hours. Invariably, subsequently, the hay-like flavor developed in the skimmilk containing added vitamin A, but not in the skimmilk without it. The hay-like flavor that developed under this condition was similar to that obtained when the vitamin A fortified skimmilk was exposed to sunlight.

3. *Increased copper content of skimmilk.* In the presence of added copper ions (cupric sulfate pentahydrate) at levels of 0.5, 1, and 1.5 p.p.m. a typical oxidized flavor developed in the skimmilk either with or without added vitamin A.

TABLE 1

Effect of sunlight on the rate of development of hay-like flavor in vitamin A fortified skimmilk[a]

Vitamin A added	Period of exposure to sunlight	Time in hours after exposure to sunlight							
		24	48	72	96	120	144	168	192
(U.S.P. units/qt)	*(minutes)*								
2,000	2	+		++	++				
2,000	0	−		−	−				
0	0	−		−	−				
2,000	2	−	+	+	+	++	++	+++	+++
2,000	0	−	−	−	−	−	−	−	−
0	0	−	−	−	−	−	−	−	−
2,000	2		+		+++	+++	+++	+++	
2,000	0		−		−	−	−	−	
0	0		−		−	−	−	−	
2,000	2	−	+	+	+	++		++++	
2,000	0	−	−	−	−	−		−	
0	0	−	−	−	−	−		−	

[a] Intensity of hay-like flavor is designated by the following: − none; ± suspected; + slight; ++ definite; +++ strong; ++++ very strong.

Usually the oxidized flavor developed in the skimmilk after 48 hours storage. Subsequently, the typical hay-like flavor then developed in the skimmilk containing the added vitamin A. Usually a much longer period was necessary to obtain the same effect with added copper than by exposure to sunlight. The results of the experiments with added copper are given in Table 2. For some reason the hay-like flavor did not seem to develop in the skimmilk when ferrous ions at levels up to 10 p.p.m. were added, even after 192 hours storage, although an intense oxidized flavor developed.

TABLE 2

Effect of added copper ions on the rate of development of hay-like flavor in vitamin A fortified skimmilk[a]

Cu	Vitamin A added	Time in hours after addition of copper							
		24	48	72	96	120	144	168	192
(p.p.m.)	*(U.S.P. units/qt)*								
0	2,000	−	−	−	−	−	−	−	−
0	0	−	−	−	−	−	−	−	−
0.5	2,000	−			oo	oo	oo	ooo	ooo++
0.5	0	−			oo	oo	oo	ooo	ooo
1.0	2,000	−		oo		ooo+	ooo++	ooo++	ooo++
1.0	0	−		oo		ooo	ooo	ooo	ooo
1.5	2,000	−	o	ooo	ooo	ooo+	ooo++	ooo++	ooo+++
1.5	0	−	o	ooo	ooo	ooo	ooo	ooo	ooo

[a] Intensity of oxidized and hay-like flavor is designated by the following symbols:

−	no flavor change	±	suspected hay-like flavor
o	slight oxidized flavor	+	slight hay-like flavor
oo	definite oxidized flavor	++	definite hay-like flavor
ooo	strong oxidized flavor	+++	strong hay-like flavor

4. *Ascorbic acid content of the skimmilk.* When skimmilk in clear glass bottles was exposed to sunlight for 2-3 minutes, a greater loss of ascorbic acid occurred in it within 24 hours than in comparable milk not exposed to radiation. The possible preventive effect of added ascorbic acid on the development of the hay-like flavor in skimmilk fortified with vitamin A was therefore determined. The skimmilk used in the experiments had about 12 mg. ascorbic acid per quart after pasteurization. Sufficient ascorbic acid was added to two lots of such skimmilk (A and B) to establish levels of approximately 40-45 and 25-30 mg. per quart, respectively. Two additional lots (C and D) were included in the series, but to these no ascorbic acid was added initially. After the elapse of the first 24 hours and at each 48-hour interval thereafter, the ascorbic acid content of each lot was measured and then restored to levels of approximately 40, 25, 10, and 0 mg. per quart, respectively *(1)*. At each 24-hour interval the milk was examined for flavor characteristics. The skimmilk with added vitamin A but containing no added ascorbic acid, exposed to sunlight, acquired the hay-like flavor in about 72 hours. The skimmilk containing the vitamin A and the added ascorbic acid and exposed to sunlight developed the hay-like flavor only after intervals from 120 to 192 hours, depending upon the concentration of ascorbic acid. An example of the results of several series of such experiments is presented in Figure 1. The added ascorbic acid definitely inhibited the development of the hay-like flavor. Riboflavin, when similarly tested, was without effect in accelerating or inhibiting the development of this flavor *(7)*.

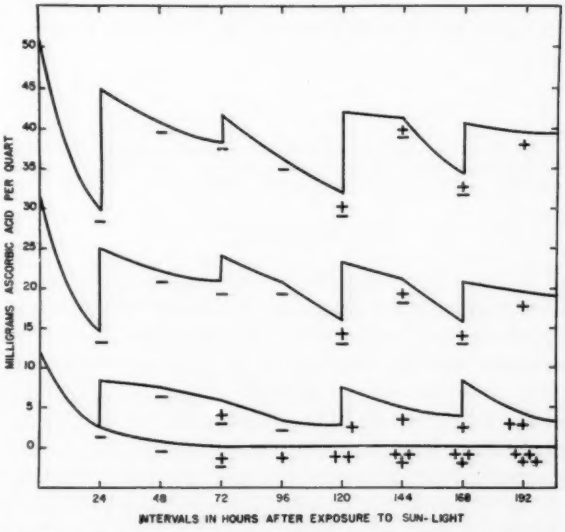

FIG. 1. Effect of level of vitamin C on the rate of development of hay-like flavor in vitamin A fortified skimmilk. Intensity of hay-like flavor is designated by the following symbols: − none; ± suspected; + slight; ++ definite; +++ strong; ++++ very strong.

5. *Addition of antioxidants.* It was observed in many instances that fairly high heat processing treatments of the skimmilk, such as to cause a slight cooked flavor, resulted in some delay in the rate of development of the hay-like flavor. The inhibitory effect of the antioxidants NDGA (nordihydroguiaretic acid) and BHA (butylated hydroxyanisole) was investigated. These were added to vitamin A fortified (2,000 I.U. per quart) skimmilks at levels of 0.1-10 p.p.m. These milks were exposed to sunlight and stored in the dark at 40° F. The NDGA was not as effective as BHA, although both definitely delayed development of the flavor. The hay-like flavor developed in the vitamin A fortified milk without BHA in 72 hours but not in the milk containing 0.1 p.p.m. BHA until after 192 hours storage. The effect of the added antioxidants is shown by the data presented in Table 3.

TABLE 3

Effect of added antioxidant on the rate of development of hay-like flavor in vitamin A fortified skimmilk[a]

NDGA in vitamin A fortified milk	Time in hours after exposure to sunlight							
	24	48	72	96	120	144	168	192
(p.p.m.)								
10.0	−	−	±	±	±	±	+	+
1.0	−	±	++		++	++	++	+++
0.1	±	±	++	++	+++	+++	+++	+++
0	±	++	+++	+++	+++	+++	++++	++++
BHA in vitamin A fortified milk	**Time in hours after exposure to sunlight**							
	24	48	72	96	120	144	168	192
(p.p.m.)								
10.0	−	−	−	−	−	−	−	−
1.0	−	−	−	−	−	−	−	−
0.1	±	−	−	−	−	±	−	−
0	−	±	++	+++	+++	+++	+++	+++

[a] Intensity of hay-like flavor is designated by the following: − none; ± suspected; + slight; ++ definite; +++ strong; ++++ very strong.

DISCUSSION

The typical hay-like flavor appears to be definitely associated with the presence of added vitamin A in the milk, since it did not occur in nonfortified milk. The characteristics of the flavor development and the manner in which it can be inhibited indicate that the reaction is of oxidative character. It has been cited that vitamin A is especially susceptible to oxidation when unsaturated fats are present under oxidizing conditions because of the catalyzing effect of the peroxides that are formed in the oxidation of the fat *(8)*. In the experiments, and in practice, marked variations occur in the rate at which the flavor develops. The hay-like flavor was observed in some milks in as short a period as 24 hours after a very brief exposure to sunlight, but in other cases 120 hours elapsed. The exposure of the vitamin fortified milk to sunlight for only a few minutes induces a trigger-like effect, following which the flavor tends gradually to

develop. Differences in sun radiation intensity, which is not constant, may account for some of the variations observed in the rate of this flavor development in different milks. The use of amber bottles was found very effective, commercially, in minimizing the development of this flavor. The effect is easily demonstrated experimentally. The radiation transmittance properties of amber glass were shown by Herreid *et al.* *(5)* to be considerably less than those of clear glass. It is possible that varying antioxidative properties of milk have some effect on the rate of development of the flavor. The survey indicated that the flavor defect occurred less frequently in milk when higher pasteurization temperatures were used and antioxidant properties presumably induced. The flavor defect is less frequently observed commercially when the milk is better protected from light, as when packaged in paper or amber glass bottles. The defect has been observed commercially in vitamin A fortified whole milk, though less frequently than in low-fat or skimmilk, primarily because there is less of it on the market. The flavor defect appears to be associated with the various forms of the added vitamin A, rather than with the forms naturally present in milk fat, since it is otherwise uncommon.

CONCLUSIONS

The factor found most responsible for the development of a haylike flavor in vitamin A fortified milk is light, especially sunlight. The best means of preventing the development of the undesirable flavor are: *(a)* the protection of the fortified vitamin A milk from sunlight or fluorescent light at all times, both paper and amber glass containers being very helpful, and *(b)* the addition or retention of ascorbic acid in the milk and added or induced antioxidant properties of the milk.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the Nopco Chemical Company for the provision of funds in support of this study.

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THE RELATIONSHIP BETWEEN FERTILITY AND THE NUMBER OF SPERMATOZOA INSEMINATED

R. W. BRATTON, R. H. FOOTE, AND C. R. HENDERSON
Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

It was early recognized *(12, 13, 14, 17)* that the number of spermatozoa inseminated could be a limiting factor in fertility. The approach to the problem then was to compare the fertility of semen diluted at different rates without regard to the variations in the number of, or the per cent of, motile spermatozoa in the semen samples, except as certain minimum standards for these and other criteria were used as a basis for discarding ejaculates. Consequently, the use of a standard dilution rate resulted in extended semen varying in motile spermatozoa numbers depending upon the concentration and per cent of motile spermatozoa in the fresh unextended semen sample.

The authors have found only three reports in the literature *(3, 4, 11)* in which fertility has been measured when predetermined and constant numbers of spermatozoa were inseminated. Two of these reports were based on the number of total, rather than the number of motile, spermatozoa inseminated.

Until the relationship between fertility and the number of spermatozoa inseminated is reasonably well established for a general set of operational conditions the genetic and net economic gains to be achieved by inseminating fewer spermatozoa than is now done cannot be reliably estimated.

The results of an experiment designed to estimate the relationship between fertility and the number of motile spermatozoa inseminated are reported here.

EXPERIMENTAL PROCEDURE

At the time the experiment was started (June 1951), it appeared from the literature *(2, 12, 13, 15, 16, 17, 18)* that the insemination of either 5×10^6, 10×10^6, or 15×10^6 motile spermatozoa per cow would probably bracket that number below which the fertility level would rapidly decrease.

Seventy-six semen samples from 20 Holstein and 16 Guernsey bulls owned by the New York Artificial Breeders' Cooperative, Inc., were used. These samples were divided into three portions and extended to either 5×10^6, 10×10^6, or 15×10^6 motile spermatozoa per milliliter of extended semen. The extender was composed of equal parts of a 2.9% sodium citrate dihydrate buffer containing 0.6% sulfanilamide and fresh egg yolk *(5)*. Each milliliter of final extender contained 500 units each of penicillin and streptomycin *(8)*.

The number of spermatozoa per milliliter of fresh unextended semen and the per cent of motile spermatozoa were determined by the routine procedures of this laboratory *(1, 14, 19)*. The semen was cooled and processed according to the procedure reported previously by Foote and Bratton *(7)*.

The breedings were made by technicians affiliated with the New York Arti-

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ficial Breeders' Cooperative, Inc. The technicians were divided into three groups and each week of a 3-week experimental period all technicians in a particular group received one of the three levels of spermatozoa numbers. The design of the experiment is shown in Table 1.

TABLE 1
Design of experiment

Week	Technician group		
	1	2	3
1	10×10^6	15×10^6	5×10^6
2	5×10^6	10×10^6	15×10^6
3	15×10^6	5×10^6	10×10^6

Fertility was estimated from the number of first service cows not returning for reinsemination within 60 to 90 days after the month in which they were inseminated.

RESULTS AND DISCUSSION

The average percentages of motile spermatozoa during 4 days of storage at 5° C. for each level of spermatozoan numbers inseminated are shown in Table 2.

The time at which most inseminations were made resulted in the use of 1- to 2-day old semen. As a consequence the numbers of motile spermatozoa inseminated were estimated to be 4.7×10^6, 9.5×10^6, and 14.3×10^6 instead of 5×10^6, 10×10^6, and 15×10^6, the number per milliliter at the time the semen was shipped from the bull stud.

TABLE 2
Average percentages of motile spermatozoa in semen samples stored for 4 days at 5° C.

Days stored	Spermatozoa levels		
	5×10^6	10×10^6	15×10^6
0	68	69	69
1	65	67	67
2	58	61	62
3	52	59	59
4	47	54	57

The best estimate *(9)*[1] of the 60- to 90-day per cent nonreturns was 66.7% for 4,111 first services in the 4.7×10^6 group, 70.9% for 4,135 first services in the 9.5×10^6 group, and 70.5% for 4,312 first services in the 14.3×10^6 group.

The average sampling standard deviation between the estimates for two treatments was 1.03 percentage units. From this it is obvious that the nonreturn rate for the 5×10^6 motile sperm extension rate is significantly lower ($P < 0.05$) than the nonreturn rates for 10×10^6 and 15×10^6.

In Figure 1 is shown the curves of relationship for the 28- to 35-day and the 60- to 90-day per cent nonreturns for the estimated number of spermatozoa inseminated. The solid lines connect those points for the actual data, and the

[1] Calculated by means of a minimum unbiased estimate method utilizing estimates of variance components.

broken lines are arbitrary projections of the solid lines through zero. These projections are presented as possible relationships between fertility and decreasing numbers of spermatozoa per insemination. They are not to be construed as the true relationships. Although a more precise description of the true curve must await more research, these projections are, in general, consistent with the results of data reported in the literature *(2, 3, 4, 5, 6, 11, 12, 15, 16, 17, 18, 20)*.

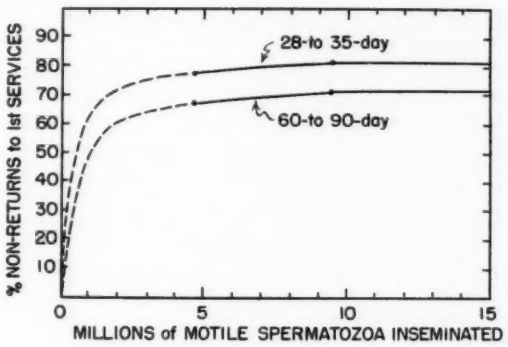

FIG. 1. The relationship of fertility to the number of motile spermatozoa inseminated.

In view of the generally high nonreturn rates for all levels of spermatozoan numbers inseminated it seems reasonable to conclude that bovine semen may be extended routinely to approximately 10×10^6 motile spermatozoa per milliliter of extended semen with the expectation of highly acceptable fertility levels. The data also indicate that 5×10^6 motile sperm per insemination will result in only a slightly lower fertility level and could result in an economic saving by reducing the number of bulls required to breed a cow population.

From a genetic point of view, decreasing by one-half the number of sires needed to breed a cow population makes possible a substantial increase in the genetic merit of the bull stud, provided adequate criteria for selection are used. In fact if there were 30 unselected, DHIA tested and artificially sired daughters of each bull, an increase in the average daughter level of approximately 12 lb. of butterfat per lactation could be expected as a result of culling the lower one-half of the bulls on the basis of these tested daughters *(10)*.

SUMMARY

Three groups of approximately 4,100 first service dairy cows each were inseminated artificially with three different concentrations of motile spermatozoa, estimated to be 14.3×10^6, 9.5×10^6, and 4.7×10^6; the 60- to 90-day per cent nonreturns were 70.5, 70.9, and 66.7, respectively.

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EFFECTS OF VARIOUS DIETS ON THE DIURNAL PATTERNS OF BLOOD PLASMA LIPIDES OF DAIRY CALVES[1, 2]

H. A. RAMSEY, G. H. WISE, S. B. TOVE, AND R. K. WAUGH
Department of Animal Industry, North Carolina State College, Raleigh

Numerous investigations have been conducted to ascertain the physiological responses of calves to various lipides that have been considered as potential substitutes for milk fat in the diet. Concentrations of blood-plasma lipides of calves fed different fats and oils vary widely, depending on many factors, among which are the particular kind of fat or oil fed and the time of collecting the blood in relation to the time of feeding. Barker and Jacobson *(2)* observed that plasma fat of calves fed reconstituted milk containing crude soybean oil, hydrogenated soybean oil, or butter oil increased to a maximum level during the first 6 hours after feeding and subsequently receded to the initial level. Huff *(13)*, on the other hand, found that the plasma fat of calves fed a semi-synthetic milk containing hydrogenated cottonseed oil rose to an initial peak at 1 hour after feeding, declined, increased to a second peak by the ninth hour, and subsequently receded to the initial level.

These differences in patterns suggested that the diurnal trends of plasma lipides might be affected by the composition of the diet. Since the kinds of fats and carbohydrates used in each of the foregoing investigations were different, a study, involving two trials, was conducted to ascertain the effects of these dietary variables on the diurnal pattern of blood-plasma lipides in dairy calves.

TRIAL I

PROCEDURES

Experimental diets. Four semi-synthetic milks containing the combinations of fats and carbohydrates shown in Table 1 were fed. Milk fat (butter) and hydrogenated cottonseed oil were selected because: *(a)* they are satisfactory sources of dietary fat for the dairy calf *(13, 15)*; *(b)* they differ considerably in fatty-acid composition *(10, 12)*, and *(c)* they are associated with differences in the diurnal trends of plasma fat of dairy calves *(2, 13)*. Lactose was chosen as one of the dietary carbohydrates, inasmuch as galactose increased fat utilization, whereas glucose, the other dietary carbohydrate chosen, did not, according to Richter *(21)*.

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[1] Approved by the Director of Research for publication as Paper No. 571 in the Journal Series.

[2] Part of the data presented in this paper is from a thesis submitted by the senior author, a Ralston Purina Research Fellow, to the graduate faculty of North Carolina State College in partial fulfillment of the requirements for the degree of Master of Science in the field of Animal Nutrition.

TABLE 1

Composition of test diets fed to experimental calves (Trial I)

Diet	Experimental variables: Fat: Milk fat[a]	Fat: Hydrogenated cottonseed oil[b]	Carbohydrate: Lactose	Carbohydrate: Glucose	Basal ingredients: Protein[c] (casein)	Minerals[d] (mixture)	Vitamins	Water
	(%)	(%)	(%)	(%)	(%)	(%)		(%)
A	—	3.50	—	6.05	4.00	1.25	*	84.95
B	—	3.50	5.50	—	4.00	1.25	*	85.50
C	4.38	—	—	6.05	4.00	1.25	*	84.07
D	4.38	—	5.50	—	4.00	1.25	*	84.62

[a] Butter containing 80% fat.

[b] Data on the degree of hydrogenation were not available. The iodine number of a previous lot of hydrogenated cottonseed oil from the same source was 72.5.

[c] Commercial grade.

[d] Per cent composition of mineral mixture: $Ca(CH_3COO)_2 \cdot H_2O$, 43.22; K_2HPO_4, 27.20; NaCl (iodized, 0.01 per cent KI), 14.13; $CaHPO_4 \cdot 2H_2O$, 8.01; $MgSO_4 \cdot 7H_2O$, 6.07; $FeSO_4 \cdot 2H_2O$, 0.84; $CuSO_4$, 0.42; $CoCl_2 \cdot 6H_2O$, 0.05; $MnCl_2 \cdot 4H_2O$, 0.03; $Zn(CH_3COO)_2 \cdot 2H_2O$, 0.03.

[e] Vitamins per 100 lb. of diet: choline chloride, 12.5 g.; inositol, 2.0 g.; para amino-benzoic acid, 1.5 g.; alpha tocopherol, 1.0 g.; niacin, 1.0 g.; calcium pantothenate, 200 mg.; menadione, 100 mg.; thiamine hydrochloride, 100 mg.; pyridoxine hydrochloride, 100 mg.; riboflavin, 100 mg.; folic acid, 50 mg.; biotin, 10 mg. In addition, each calf received 20,000 I.U. of vitamin A and 1,500 I.U. of vitamin D daily.

The diets were mixed and processed according to the following procedure: Sodium bicarbonate equal to 4.75% of the casein was added to water at a temperature of 140° F. The casein was added slowly to the sodium bicarbonate solution and stirred thoroughly for 20 to 30 minutes. The carbohydrate and the melted fat were added successively. Each sugar was included in the respective diets in equimolar concentrations on a monosaccharide basis. The mixture was homogenized at a pressure of 2,500 p.s.i. and then cooled. Minerals (previously dissolved in hot water) and vitamins were added. The diets were prepared at weekly intervals and were stored at 37° F. until fed.

Experimental design. The test diets were fed to each of four male Holstein calves in accordance with a Latin square design (Table 2), in which a given diet was preceded by any other diet only once.

The primary criteria of effects were the diurnal patterns of several lipide fractions (Allen's "plasma fat," total lipides, lipide phosphorus, and total cholesterol) in the blood plasma and of sugar in the whole blood.

TABLE 2

Schedule of feeding test diets to experimental calves (Trial I)

Calves	Diets fed during the consecutive 2-week feeding periods: I	II	III	IV
H-1	B	A	D	C
H-2	A	C	B	D
H-3	D	B	C	A
H-4	C	D	A	B

A = Hydrogenated cottonseed oil and glucose diet.
B = Hydrogenated cottonseed oil and lactose diet.
C = Milk fat and glucose diet.
D = Milk fat and lactose diet.

Feeding and management of experimental animals. The calves, after receiving colostrum from their dams for several days, were assigned to their respective test diets. These diets were fed from nipple pails at 12-hour intervals, 7 A.M. and 7 P.M. The daily rate of feeding was 1 lb. of diet per 10 lb. of body weight. Calves were weighed on alternate days so that the allocations of milk could follow closely the weight gains. The animals were muzzled to prevent consumption of shavings used for bedding.

Collection and analysis of blood samples. A series of blood samples was drawn from the jugular veins of each calf on the seventh and the fourteenth day of each 2-week feeding period. The initial sample was collected immediately before feeding (7 A.M.) and subsequent samples, at hourly intervals through the twelfth hour. Sodium oxalate was used as the anti-coagulant.

The procedure of Allen *(1)* was used to determine "plasma fat," which approximates the sum of neutral fat, free cholesterol, and cholesterol esters *(7)*.

An alcohol-ether extract of the plasma, prepared as outlined by Folch and Van Slyke *(11)*, was used for the determinations of total lipides and of lipide phosphorus. For total lipides, an aliquot of the alcohol-ether extract containing between 100 and 300γ of lipide material was placed into a small test tube. The alcohol and ether were removed by evaporation in a steam-heated oven. The sodium dichromate reducing capacity of the residual lipide material was determined according to the method of Johnson *(16)*. Application of this method to blood-plasma lipides of calves revealed that an average of 3.98γ of lipide material were oxidized by 1 μeq. of sodium dichromate. This factor was used in computing the total-lipide values.

Lipide phosphorus was determined by placing a second aliquot of the alcohol-ether extract, containing between 50 and 100γ of phosphorus, in a 25 $\times$ 200 mm. Pyrex test tube calibrated at 15 ml. After removal of the alcohol and ether by evaporation, the residual lipides were wet-ashed by adding 1.5 ml. of a mixture of concentrated nitric and perchloric acids (2:1) and heating in a sand bath to near dryness. Approximately 10 ml. of boiling distilled water were added. After increasing the volume to 15 ml. by addition of distilled water at room temperature, 1.5 ml. of a combined vanadate-nitric acid reagent *(23)* and 1.5 ml. of a 5% aqueous ammonium molybdate solution were added successively. The contents of the test tube were mixed by shaking and permitted to stand 20 to 30 minutes to insure complete development of color. The yellow molybdivanadophosphoric acid formed was estimated against appropriate standards in an Evelyn colorimeter, using a 420 mμ filter.

Total cholesterol was determined according to the method of Saifer and Kammerer *(22)*. Blood sugar was estimated by means of the procedure presented by Nelson *(19)*. Glucose was used as the reference standard.

RESULTS

The results from Trial I are summarized graphically in Figures 1, 2, and 3. Statistical analyses of the experimental data revealed no significant differences, ascribable to dietary factors, in the diurnal patterns of total lipides, lipide phos-

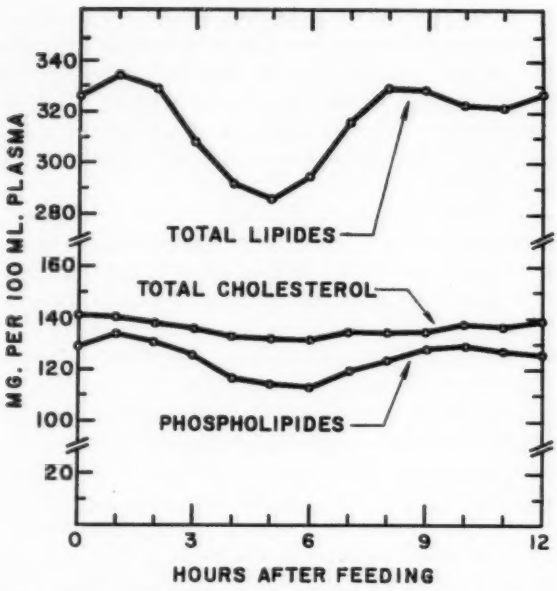

FIG. 1. Mean diurnal patterns (average of data from all diets) of total lipides, total cholesterol and phospholipides (lipide phosphorus × 25) in blood plasma of dairy calves.

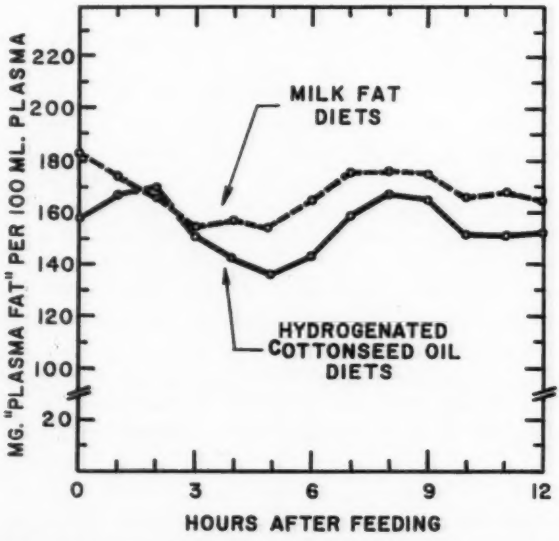

FIG. 2. Mean diurnal patterns of Allen's "plasma fat" in blood plasma of calves receiving test diets containing hydrogenated cottonseed oil and milk fat, respectively.

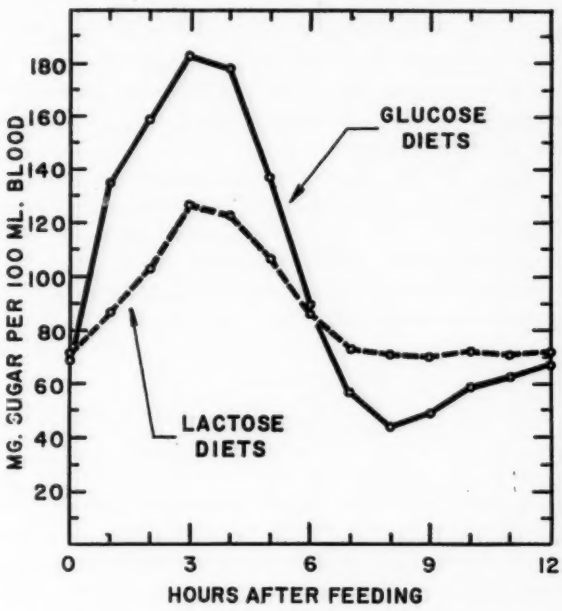

FIG. 3. Mean diurnal patterns of blood sugar of calves receiving test diets containing glucose and lactose, respectively.

phorus, or total cholesterol. Therefore, the respective curves in Figure 1, representing patterns of these three plasma lipide fractions, were derived from the averages of data from all diets. To facilitate comparison with the other lipide fractions, lipide phosphorus is presented as phospholipides (lipide phosphorus $\times 25$).

The mean diurnal patterns for total lipides and phospholipides are similar. There was a slight to moderate increase during the first 2 hours, a decline to below the initial level by the fifth hour, a subsequent increase to a peak by the eighth or ninth hour, and a recession to approximately the initial level by the twelfth hour. The level of total cholesterol decreased slightly during the first 6 hours and then returned to the initial level.

The mean diurnal patterns of "plasma fat" associated with the hydrogenated cottonseed-oil diets (Figure 2) are similar to those previously reported *(13)* but are different ($P < 0.01$) from those of the milk-fat diets during the first 3 hours after feeding. During this interval, "plasma fat" from the hydrogenated cottonseed-oil diets increased to a peak by the second hour and subsequently decreased to below the initial level, whereas "plasma fat" from the milk-fat diets started to decrease immediately after feeding. After the fifth hour, the diurnal patterns of "plasma fat" from the respective dietary fats were similar, resembling those of total lipides (Figure 1).

Blood sugar concentrations from the glucose diets increased to a higher level during the first 3 hours after feeding than did those from the lactose diets (Figure 3). After the third hour, blood sugar from the former diets dropped precipitously to a level below the initial and returned gradually to this level, whereas blood sugar from the latter diets receded slowly to the initial level and remained almost constant thereafter.

The kind of fat in the diet seemed to have no effect on the diurnal patterns of blood sugar, and the kind of carbohydrate present seemed to have no effect on the diurnal pattern of "plasma fat." The initial peak in "plasma fat" from the hydrogenated cottonseed-oil diet containing lactose was less pronounced, however, than that for the same fat diet containing glucose, although this difference was not statistically significant.

TRIAL II

When the semi-synthetic diets containing milk fat were fed, the diurnal pattern of "plasma fat" (Figure 2) was unlike that noted when a reconstituted nonfat-milk-solids diet containing butter oil was fed *(2)*. These apparent differences in responses from diets that contained the same kind of fat and of carbohydrate evoked the possibility that some other property or constituent of the diet may be involved. Trial II, therefore, was initiated in an effort to explore other probable etiological factors.

PROCEDURES

Two test diets were chosen; one was identical with diet B (hydrogenated cottonseed oil and lactose) fed in Trial I, but the other contained nonfat milk solids in lieu of the casein, lactose, and mineral mixture of diet B. Mixing and processing of the test diets were the same as for Trial I except that no sodium bicarbonate was required to dissolve the nonfat milk solids.

TABLE 3

Schedule of feeding test diets to experimental calves (Trial II)

Calves	Diets fed during the consecutive 2-week feeding periods			
	I	II	III	IV
H-5	E	E	E	E
H-6	B	B	B	B
H-7	B	E	B	E
H-8	E	B	E	B

B = Semi-synthetic diet (hydrogenated cottonseed oil and lactose).
E = Nonfat milk solids diet.

The two test diets were fed to four male Holstein calves, beginning at 2 days of age and continuing for 8 weeks according to the schedule in Table 3. The respective test diets were fed continuously to two calves throughout the trial, whereas the test diets for the other two calves were alternated at 2-week intervals. Feeding and management of these animals and collection of blood samples

were identical to the procedures used in Trial I. The analysis of the blood plasma, however, was restricted to Allen's "plasma fat" *(1)*.

RESULTS

The feeding schedule per se, alternate or continuous, seemed to have little or no effect on the diurnal patterns observed. Therefore, the curves presented in Figure 4 represent averages of all the data from each diet without regard to the order of feeding. A marked difference between the two test diets was readily evident during the first 8 hours.

The level of "plasma fat" following feeding of the semi-synthetic diet decreased during the first 4 hours and then rose to a peak at about the eighth hour. Following feeding of the nonfat-milk-solids diet, however, the level of "plasma fat" increased during the first 3 hours, dropped slightly for an hour and then continued to increase until the seventh hour. After the eighth hour, the trends for both diets appeared to be the same. The pattern for the semi-synthetic diet was similar to that observed for the hydrogenated cottonseed-oil diets in Trial I (Figure 2) except that the peak during the initial stage was not manifested. This difference may be related to the fact that lactose was fed instead of glucose.

DISCUSSION

Changes in the concentration of lipides in blood plasma depend on alterations in the relative rates of two opposing factors, the addition of lipides to and the removal of lipides from the plasma. An increase in the level of plasma lipides occurring immediately after the ingestion of fat generally is regarded as evidence that fat is being absorbed. On the other hand, a decrease in the level of plasma lipides under similar circumstances might result from *(a)* a temporary delay or depression in fat absorption or *(b)* a normal rate of fat absorption accompanied by a greatly accelerated rate of lipide removal from the plasma.

The diurnal pattern of "plasma fat" for the nonfat-milk-solids diet in Trial II (Figure 4) was similar not only to that observed previously for calves *(2)* but also to those of blood lipides for other species *(4, 14, 24, 26)*. The fact that the level of "plasma fat" continued to increase for several hours after feeding suggests that fat absorption was taking place during this interval.

The results of Trial I (Figure 2) indicate that the diurnal pattern of "plasma fat" may be modified slightly by the kind of fat in the diet. In view of the difference in responses from the semi-synthetic diet and the nonfat-milk-solids diet in Trial II (Figure 4), there can be little doubt that dietary factors other than the kind of fat also were involved. The factors in the semi-synthetic diets contributing to the decrease of "plasma fat" to below the initial level after feeding and the manner in which the observed effects were produced cannot be ascertained from the present observations.

The observed decrease and subsequent increase might have been the result of initial retardation of absorption followed by normal or accelerated absorption. One possible cause of a period of delayed absorption could be a slow passage of

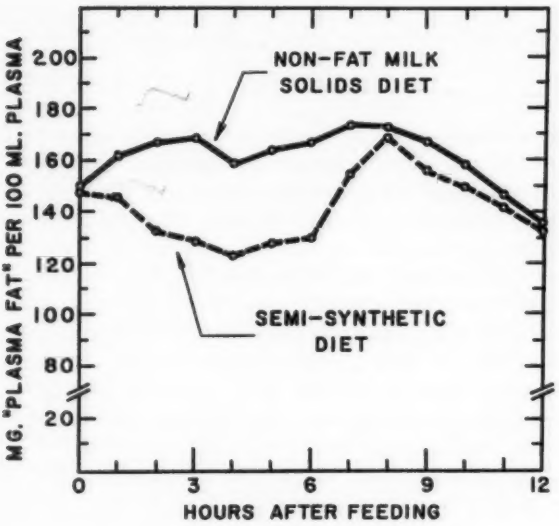

FIG. 4. Mean diurnal patterns of Allen's "plasma fat" in blood plasma of calves receiving nonfat milk solids and semi-synthetic test diets, respectively.

fat from the stomach. Gastric emptying-time in calves has been shown to be affected by the curd tension of milk *(8, 9, 18)*. Soft curd passed from the abomasum more rapidly than did hard curd. Curd tension was not measured while Trials I and II were in progress, but subsequent measurements, according to a standard procedure *(17)*, revealed that the curd tension of diet B (10-14 g.) was slightly higher than that of the nonfat-milk solids diet (5-7 g.). Since both diets had curd tensions sufficiently low to be classed as "soft curd" milks (< 30 g.), the foregoing difference is difficult to evaluate in terms of its effect on gastric emptying-time.

A second possibility for the initial decrease in "plasma fat" might exist in the calcium-magnesium and fat absorption interrelationship *(3, 5, 6)*. The general observations have been that with high levels of calcium and magnesium intake the absorption coefficients of dietary fats frequently are reduced and that with high levels of fat in the diet the total retention of calcium often is lowered. These phenomena have been ascribed primarily to the formation of relatively insoluble calcium and magnesium soaps of the higher saturated fatty acids and the accompanying reduction in total absorption. Since the semi-synthetic diets fed in this investigation contained forms of calcium and magnesium that may be readily available for the formation of soaps within the intestinal lumen as the fat undergoes lipolysis, such saponification warrants consideration as a factor affecting absorption.

Another possibility that might account for a delay in fat absorption is the temporary inhibition of lipolysis by some dietary ingredient. The tenableness

of such speculation depends on the assumption that lipolysis is an important step in the process of fat absorption by the calf. Weinstein and Wynne *(25)* observed that in vitro activity of swine pancreatic lipase was inhibited markedly in the presence of small concentrations of cupric, ferrous, ferric, and cobaltous ions. Similar inhibition of calf pancreatic lipase by cupric and cobaltous ions also has been noted *(20)*. The concentrations of ferrous and cupric ions in the semi-synthetic diets fed in Trials I and II were far in excess of those required to produce appreciable in vitro inhibition of pancreatic lipase and in greater concentrations than those probably present in the nonfat-milk-solids diet. The extent to which the foregoing in vitro findings may be applied to in vivo conditions in the calf is difficult to ascertain. Nevertheless, it is possible that immediately after feeding, the concentrations of the ferrous and the cupric ions in the diet were sufficiently high to depress lipolytic activity to such an extent that the quantity of fat absorbed was not adequate to maintain a constant blood lipide level. Subsequently, these ions might have been absorbed, complexed in some inactive form, or passed along the intestinal tract more rapidly than the fat, thus creating a reduction in their effective concentration and permitting resumption of sufficient lipolytic activity for fat absorption to proceed at a normal rate.

In view of the fact that semi-synthetic diets are being used increasingly as a tool in the study of calf nutrition, the manner in which such diets appear to modify fat absorption merits further investigation.

SUMMARY

The effects of semi-synthetic diets containing different fats (hydrogenated cottonseed oil or milk fat) and carbohydrates (lactose or glucose) on the diurnal patterns of various blood plasma lipides (Allen's "plasma fat," total lipides, total cholesterol and lipide phosphorus) and blood sugar of dairy calves were determined.

Of the patterns studied, differences in dietary fat were reflected only in trends of "plasma fat" and only during the first 5 hours after feeding. "Plasma fat" of calves fed the milk-fat diets decreased until the third hour and plateaued to the fifth hour, whereas that of calves fed the hydrogenated cottonseed-oil diets increased to a peak by the second hour and declined to the fifth hour.

The diurnal patterns for total lipides and lipide phosphorus were similar and, in general, were characterized by a slight to moderate increase during the first 2 hours, a decline to below the initial level by the fifth hour, a subsequent increase to a peak by the eighth or ninth hour, and a recession to approximately the initial level by the twelfth hour. The level of total cholesterol decreased slightly during the first 6 hours and then returned to the initial level.

The kind of dietary carbohydrate affected only the diurnal pattern of blood sugar. Blood sugar in calves fed glucose increased more rapidly, reached a higher peak, declined more precipitously and came to equilibrium more slowly than in calves fed lactose.

The diurnal patterns of "plasma fat" associated with the feeding of diets

different in the nonfat components but the same in kind of fat (hydrogenated cottonseed oil) differed markedly. After feeding the diet with the "nonfat-milk-solids" base, "plasma fat" increased to a maximum level during the first 7 hours and subsequently receded. After feeding the diet with the synthetic base (lactose, casein, and minerals), however, "plasma fat" decreased to below the initial level during the first 5 hours, increased to a peak at the eighth hour, and then receded. Several factors that may be related to the different responses are presented.

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INHERITANCE OF BLACK HAIR PATTERNS IN CATTLE LACKING THE EXTENSION FACTOR FOR BLACK (E). I. BLACKISH[1]

C. S. BALDWIN,[2] L. O. GILMORE, AND N. S. FECHHEIMER

Department of Dairy Science and the Institute of Genetics
The Ohio State University, Columbus

AND

The Ohio Agricultural Experiment Station, Wooster

Blackish is the term used by Ibsen *(3)* to designate hairs found in Ayrshire and Jersey cattle that microscopically were found by Bogart and Ibsen *(1)* to contain partially extended black clumps. Therefore, both red and black show in such hairs.

Ibsen suggested the symbol *Bs* to designate the gene for these hairs and concluded that it was inherited as a single dominant and is one of two modifiers of *B*, the gene responsible for black pigment in the hair and skin.

The presence of *E*, the other modifier of *B*, has been postulated to be the gene responsible for complete extension of black pigment in all individual hairs that are pigmented *(4)*. The gene *E* acts on *B* to form closely packed clumps of black pigment throughout all pigmented hairs and skin areas.

According to Ibsen *(4)*, neither *Bs* nor *C (red)* can be expressed except in the presence of *ee*, the absence of the extension factor.

Blackish *(Bs)* is hypostatic to *E* and causes a partial concentration of black pigment in the individual hair and skin of animals that are of the composition *ee*, such as Ayrshires, most Jerseys, and Brown Swiss. Blackish does not affect all pigmented hairs but is limited, as far as now known, to certain indefinitely outlined areas.

It has been suggested by Bogart and Ibsen that the partial extension gene *(ep)* for guinea pigs and blackish *(Bs)* in cattle are similar. Whereas *Bs* affects limited areas in cattle, *ep* in guinea pigs causes the partial extension of black pigment in the hair of all areas normally composed of pigmented hair.

According to Bogart and Ibsen, *Bs* like *B* "causes black pigment to appear in the skin, hoofs, nose, tongue, lining of the mouth, eyelids and 'whites' of the eyes if the animal is a self *(8)*." This would apply particularly to the Jersey breed. The great majority of animals in which black tongue is present in the Jersey breed is considered by Ibsen to be due to the *Bs* factor *(3)*.

The object of the study herein reported was to delineate more sharply the specific manifestations of the *Bs* gene in hair, to study the effect of age on its expressivity, and to ascertain the frequency of this gene in the Ayrshire and

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[2] Present address: Central Ohio Breeding Association, Columbus, Ohio.

Jersey breeds. The publication of a similar analysis of mahogany, a modifier of blackish, will follow. Several observations on brindling, hitherto unpublished, also are recorded.

EXPERIMENTAL PROCEDURE

The animals on which the initial observations were made came from five privately owned herds and from The Ohio State University herd. It soon became evident that in spotted cattle carrying a predominant amount of white *(lw lw* and in some cases *Lw lw)* it was difficult or impossible to detect the presence of *Bs* hairs on the legs and ventral portions of the body, shoulders, and thighs. The desirability of using self *(S)* animals, therefore, became apparent. Because *Bs* was found in its various manifestations in Jerseys, observations similar to those in Ayrshires were recorded for the University Jerseys. Thereafter, observations were made on cattle of both breeds at the West Virginia University, the Ohio State Fair (1950-51), the International Dairy Exposition (1950), and Central Ohio Breeding Association. A total of 1,250 cattle from herds representing the Ayrshire and Jersey breeds was observed. This was considered to be equivalent to a random sample because there is no known widespread prejudice for or against this gene in these two breeds.

One advantage of collecting data at the fairs was the relatively small number of animals of either sex representing any one herd. Furthermore, the bulls in the show string usually included a bull of outside breeding in the case of either the senior or junior herd sire, or both. In the West Virginia University herd of Ayrshires, a definite plan for bringing in outcross bulls and using them to a limited extent has been practiced from the beginning of the experiment *(2)*. The private herds observed were small with no line breeding attempted.

All cattle were classified according to shade of red color, i.e., light, medium light, medium, medium dark, or cherry red. When it became evident that some cattle were "brown" rather than the above shades of red, this fact also was noted. Because of the relationship of brindling to both blackish and mahogany, this characteristic was likewise noted for each animal inspected. The same color classification was used in Jerseys as in Ayrshires.

A microscopic investigation of varying shades of hair color was considered fundamental in order to establish a close correlation between the macroscopic and microscopic classification of blackish hairs as described by other workers. Samples were obtained from different pigmented areas of the body. After most of the dust was removed by drawing the hair through the forefinger and thumb, permanent mounts in balsam were prepared. Photomicrographs were made of certain specimens as a means of establishing permanent references.

From the onset it was evident that considerable variation existed in the areas of the body where blackish hairs were found. Accordingly, a check list of the location of blackish hairs and other color patterns on the body was devised in order to obtain necessary data pertaining to this character and to establish a more definite idea of the manner of its expression. The locations used in this

list included the muzzle, cheek, eyes, ear, neck, shoulder, legs, body, rump, thigh, tail, and switch. The frequency with which blackish hairs appeared in each area was recorded for each age grouping.

Based on the conclusion that *Bs* is a simply inherited autosomal dominant and the assumption that no epistatic modifiers exist, the frequency of *Bs* was obtained by subtracting the square root of the recessive genotypic frequency from one.

RESULTS AND DISCUSSION

It was observed that as the variation in shade of hair deepened from a light red to dark or cherry red, the black pigment granules in the cortex apparently became more abundant and closely packed (Figure 1). It could not be determined accurately by microscopic examination whether or not the shade of red pigment appearing diffuse and nongranular varied in intensity. In light red hairs the black pigmented granules were scattered comparatively far apart, allowing the diffuse red pigment in the cortex to be plainly visible (Figure 1, lower). The structural outlines of the cuticle, cortex, and medulla are plainly visible in these red hairs, and the medulla is a solid black structure. There were, however, a few exceptions, e.g., in some hairs the medulla appeared to be discontinuous.

Cherry-red hairs were found to be macroscopically and microscopically distinguishable from blackish hairs. Microscopically, the black pigmented granules of blackish hairs are very closely clumped, making it impossible to delineate the medulla from the cortex. The red pigment in these hairs is visible, but only toward the outer edge of the cortex (Figure 2). Apparently this is due to the black pigmented granules being concentrated in the cortex nearest the medulla or the greater depth of hair involved. Macroscopically, blackish hairs appear to be a deep shade of red when held singly up to light, as compared to the black appearance when on the body of an animal.

When black hairs *(E)* were examined under the microscope, they appeared to be solid black, the cortex being indistinguishable from the medulla. By means of an oil immersion lens it was possible to locate a few areas near the outer edge of the cortex where red pigment was visible.

From these microscopic examinations of various color shades of hair, blackish hairs were found to be an intermediate variation of deep cherry-red hairs and true black hairs. Blackish *(Bs)* may be defined, therefore, as a modifier of *B* that causes black pigment concentration within the cortex of red *(ee)* cattle hairs. It differs from *E* in that *Bs* hairs appeared to contain more diffuse red pigment at the periphery of the cortex.

These observations agree closely with those of Bogart and Ibsen, but they extend the detail with which it is possible to describe this trait. The diagnosis of this trait is now possible with greater accuracy through the use of the technique described above.

Delineation of blackish phenotypes. It was necessary to establish a basic concept for the limitations and variations in the expression of this gene because

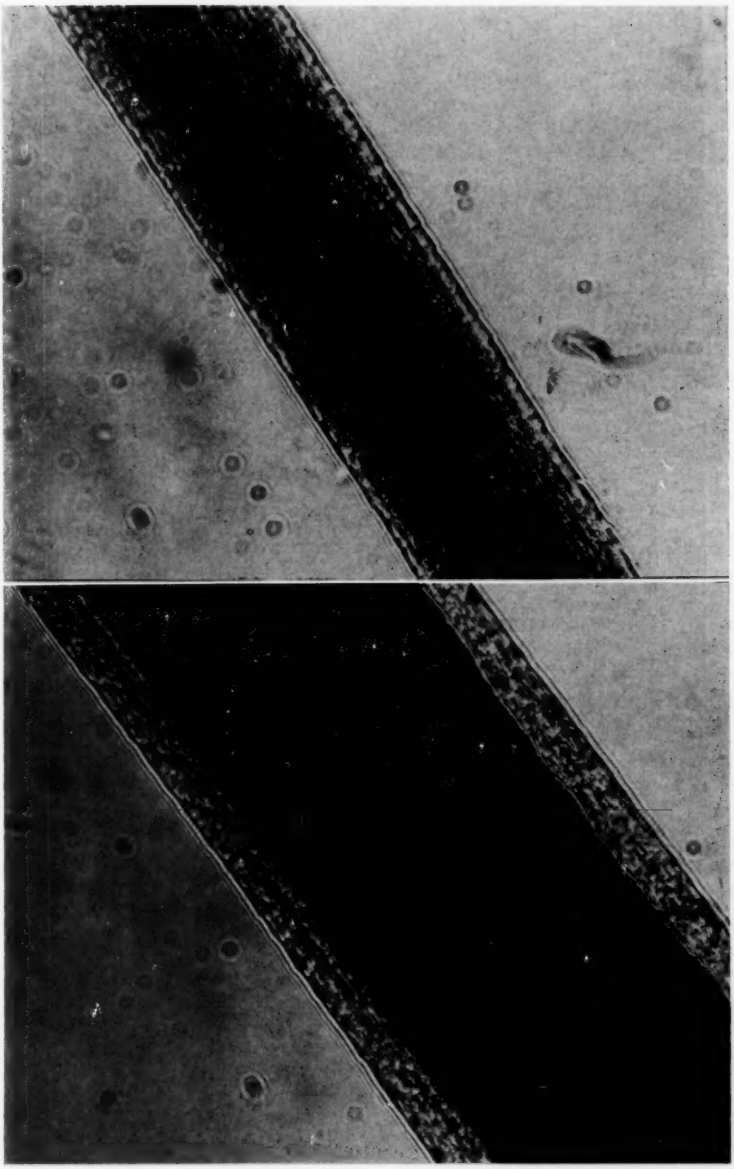

FIG. 1. Photomicrograph of whole mount of red hairs (×450). (Upper) Tip end of dark red hair showing concentrated black pigment in cortex. (Lower) Base end of a light red hair showing scattered black pigment granules in the cortex.

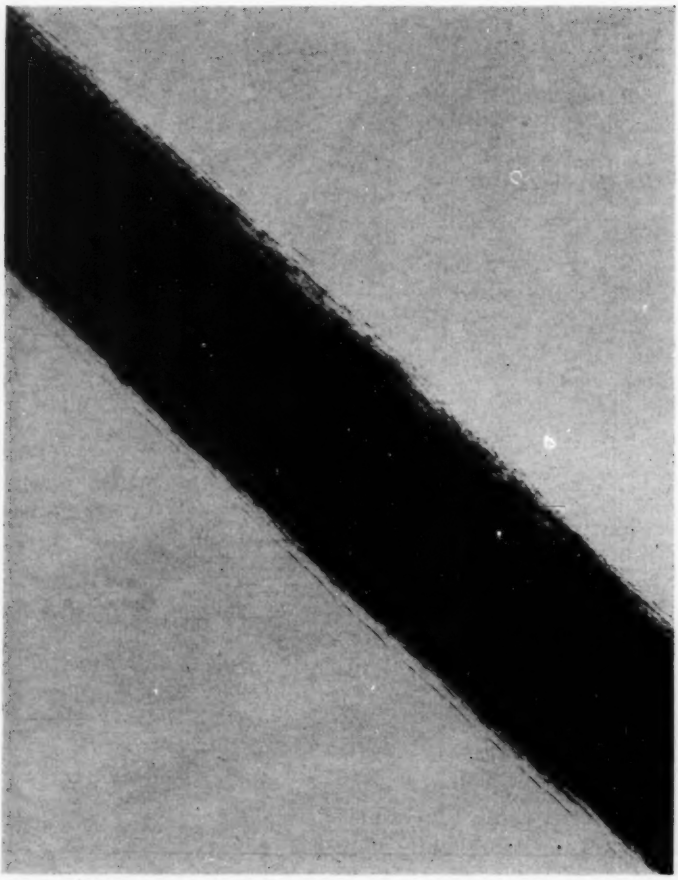

FIG. 2. Photomicrograph of whole mount of a blackish hair (×450).

of extreme variation in the location and extent of blackish hairs in cattle.

The frequency with which blackish occurred in the different locations of the body was summarized by individual age groups. The area showing consistently the highest frequency of occurrence in each age group was the area used for establishing the presence or absence of the blackish phenotype. The earliest age at which this trait was manifested in either breed was 1 month and 4 days in a Jersey heifer. Two bull calves did not develop any blackish hairs until 6 months of age.

To allow for full physiological expression of the gene with age in obtaining the frequency of *Bs*, in the sample studied all values for animals below the age of the maximum incidence are excluded. For females, this age was chosen at

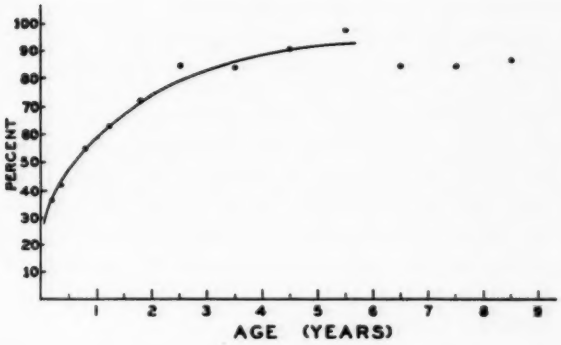

FIG. 3. Frequency of blackish with age in 1061 Ayrshire and Jersey females.

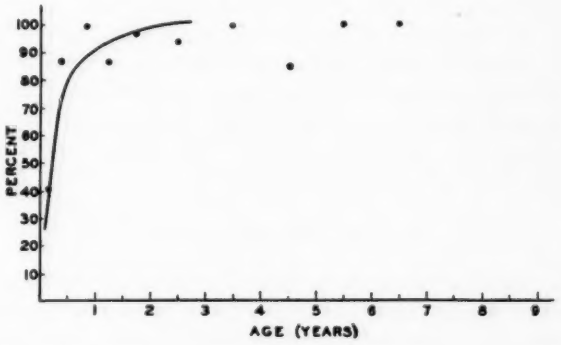

FIG. 4. Frequency of blackish with age in 189 Ayrshire and Jersey males.

2 years after a consideration of the distribution plotted in Figure 3. The age for males was chosen as 1 year (Figure 4).

With one exception, *Bs* hairs were found in areas around the eyes if they were present at all. These hairs were considered distinct from black or black-like hair in the ear, switch, and the distal region of the legs. The area of second highest frequency was the area of the cheek, i.e., the region of the maxilla and mandible, ventral to the eye. The age frequency curves for blackish hairs in the ear, switch, and distal part of the leg are not the same as those of other areas considered to have *Bs* hairs, i.e., around the eyes and cheek.

With the expression of *Bs* established in the above manner, the frequency of expression was found to increase with advancing age until 1 year of age in males and 2 years of age in females (Figures 3, 4).

Six of the 173 Jerseys, or 3.47%, which reached 2 years of age at the time of observation were found to be nonblackish *(bsbs)*. The square root of 0.035 is

0.19, which represents the frequency of the gene, *bs*. One minus 0.19 leaves 0.81 to express the frequency of *Bs* in Jerseys. Similarly, 65 of 364 Ayrshire females over 2 years of age, or 17.86%, were found to be nonblackish. Thus, the frequency of *bs* in Ayrshires for the ages studied was 0.42 and of *Bs* 0.58. These frequencies of *Bs* are valid under the assumptions set forth above, i.e., complete dominance of *Bs*, no epistatic modifiers and randomness of sampling.

Relation of brindling to blackish. Brindling has been observed in Ayrshires in all areas of the body showing blackish hairs. It has not been observed in the ear, switch, or the distal part of the leg. That brindling is a modifier of blackish and is hypostatic to *bsbs* is further demonstrated by the lack of brindling in animals that are recessive for blackish *(bsbs)* but which, nevertheless, were ascertained to transmit *Br* to their progeny.

Brindling also has been observed in Guernsey cattle, a breed for which *Bs* has not previously been postulated. Data have not been collected to show the frequency of *Bs* in this breed; however, cases of *Bs* occurring in the head (cheek and eyes) have been observed. Furthermore, it has been observed in a few instances where the expression of the *Bs* has been modified in the typical brindling pattern in the head regions of the cheek and eye. Prejudice against color markings of this type in Guernsey cattle exists, as revealed by breeders. Selection against *Bs* and *Br* would explain the low incidence in this breed. *Bs* in Guernseys is defined the same as in Ayrshires and Jerseys and is not meant to refer to the black hairs found around the muzzle.

SUMMARY AND CONCLUSIONS

Microscopic examination of hairs from Ayrshire and Jersey cattle verified the observation of Bogart and Ibsen that blackish hairs have a concentration of black granules in the cortex resulting in a shade of color intermediate between dark cherry red and black.

An analysis of the areas where blackish hairs occurred indicated that several areas were involved in different frequencies to give a great range in the expressivity of this trait. The areas most frequently affected are around the eye, muzzle, cheek, and neck. If present at all, it was found in one of the three head areas indicated.

The frequency of occurrence of blackish increases with age and it apparently reaches a maximum at approximately 2 years of age in females and 1 year in males.

Blackish was not observed in any calf at birth or during the first month thereafter. Beginning shortly after 1 month of age, there was a progressive increase in the percentage of calves in which this trait was diagnosed.

ACKNOWLEDGMENT

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RELATIONSHIP BETWEEN PLASMA VITAMIN A AND LIVER VITAMIN A IN CALVES FED A VITAMIN A DEPLETION RATION AND CALVES FED MINIMUM LEVELS OF VITAMIN A OR CAROTENE[1]

J. E. ROUSSEAU, JR., CECILIA M. DEMBICZAK, K. L. DOLGE, AND H. D. EATON
Animal Industries Department, Storrs Agricultural Experiment Station

GEOFFREY BEALL
Statistics Department, University of Connecticut, Storrs

AND

L. A. MOORE
Dairy Husbandry Research Branch, USDA, Beltsville, Md.

Since the liver has been found to be the main storage site of vitamin A in cattle *(15)*, the derivation of a quantitative relationship between plasma vitamin A concentration and liver vitamin A concentration would be of value in ascertaining vitamin A storage. Data which suggest such a relationship have been reported for steers, calves, lambs, and pigs fed varying intakes of vitamin A and/or carotene *(12, 16, 17, 20)*, steers and calves fed vitamin A depletion rations *(6, 11)*, and calves, kids, lambs, and pigs from dams fed varying intakes of carotene and/or vitamin A *(9, 23, 25, 26)*. Recently Almquist *(1)* has derived a quantitative relationship between plasma vitamin A concentration and the logarithm of liver vitamin A concentration in fowl.

In the rat, such a relationship appears controversial. Steigmann and Popper *(24)* have suggested a parallelism between plasma and liver vitamin A concentrations, and the data of others *(14, 20)* gave support to this, but Krause *(19)* has reported an inverse relationship at high liver vitamin A concentrations. Glover *et al. (14)* have reported that plasma vitamin A levels are more nearly proportional to liver vitamin A alcohol concentrations than to total liver vitamin A concentration, and Ganguly and Krinsky *(13)* have given evidence that a direct relationship was nonexistent.

The objective of this study was to determine whether a quantitative relationship existed between blood plasma vitamin A and liver vitamin A concentrations in calves fed a vitamin A depletion ration and in calves fed minimum levels of vitamin A or carotene.

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EXPERIMENTAL PROCEDURE

Animals and rations. The data reported herein were derived from 97 male calves fed either a vitamin A depletion ration (the depletion group) or the same depletion ration plus minimum levels of vitamin A or carotene (the vitamin A and carotene group). The depletion group, consisting of 15 Guernseys and 43 Holsteins, was raised to 91 or 105 days of age on a limited whole milk system and either limited starter and ad libitum hay *(7, 10)* or ad libitum fixed mixtures of artificially dehydrated alfalfa and starter or mixtures of these in which the parts of starter were held consta. and the parts of artificially dehydrated alfalfa were increased at weekly intervals *(8)*. On the 92nd or 106th day of age these calves were fed a vitamin A depletion ration *(7, 8)* adjusted at successive 7-day intervals for the weight of each calf. When a particular calf's blood plasma vitamin A level decreased to an assigned interval of 2.0γ%, it was slaughtered. Assignment to a particular blood plasma vitamin A interval was insofar as possible according to a previously randomized allotment with restriction as to breed and previous treatment. At slaughter the calves averaged 128 days of age with a standard deviation of 19 and weighed 263 $\pm$ 72 lb.

The vitamin A and carotene group, consisting of 39 Holsteins, was raised to 63 days of age on a limited whole milk, limited dry calf starter and ad libitum hay system *(22)*. On the 64th day of age the calves were fed the same vitamin A depletion ration but at an intake to give an anticipated 7-day rate of increase in body weight of 10 lb. When the blood plasma vitamin A level for each calf decreased to 10γ% or less, calves were fed either one of five levels of vitamin A from a dry carrier or one of five levels of carotene from alfalfa. These were fed at the rate of 2, 4, 6, 8, and 10γ of vitamin A or 12, 18, 24, 30, and 36γ of carotene per pound of body weight per day. The duration of feeding the vitamin A or carotene was 16 successive 7-day age periods for each calf. Upon completion of this feeding period each calf was slaughtered. At this time their average age was 200 $\pm$ 9 days and average body weight 436 $\pm$ 48 lb.

Observations and analyses. All feeds fed and refused for each calf were weighed to the nearest 0.1 lb. Venous blood samples were taken at weekly intervals, as were body weights to the nearest 1 lb. All calves were slaughtered between 7 and 10 A.M. at the end of their respective experimental periods. Either the P.M. prior to slaughter or immediately before slaughter the calf's body weight was determined and a venous blood sample was taken for vitamin A analysis *(2, 18)*. The whole liver was macerated in an Hobart food chopper, and a representative sample was taken for subsequent vitamin A analysis *(4)*. The data reported herein pertain only to the blood plasma and liver vitamin A concentrations at the time of slaughter.

The functional form used initially for the relationship of plasma vitamin A concentration on liver vitamin A concentration was $Y = a + b\ (\log X)$ in which Y = plasma vitamin A concentration and $\log X$ = logarithm of the liver vitamin A concentration *(1)*. Upon graphing the data in this form, it was found that as the plasma vitamin A concentration increased its variation also increased. There-

fore, to provide as uniform variance as possible, the plasma vitamin A values were transformed to logarithms. Standard statistical methods *(5)* were employed in the derivation of the quantitative relationship.

RESULTS AND DISCUSSION

In Table 1 are presented data pertaining to the linear regressions of the relationship of log plasma vitamin A concentration on log liver vitamin A concentration, and in Figure 1 are given the individual calf values, as well as groups' linear regression lines. Regressions of higher order than linear were not indicated, since inappreciable reduction in the variance of log plasma vitamin A occurred when second order terms were introduced.

TABLE 1

Relationship of plasma vitamin A on liver vitamin A expressed as linear functions

	No. of calves	Coefficients for the linear relationship of log plasma vitamin A concentration on log liver vitamin A concentration		Standard error of estimate	Variation in log plasma vitamin A accounted for by log liver vitamin A
		Intercept	Slope		
Calves fed a vitamin A depletion ration		*(a)*	*(b)*	*(s y.x)*	*(%)*
Guernseys	15	+0.31 ±0.12[a]	+0.3164 ±0.0552[a]	0.09	72
Holsteins	43	+0.26 ±0.07	+0.3076 ±0.0292	0.10	73
Both breeds	58	+0.30 ±0.06	+0.2964 ±0.0257	0.10	70
Calves fed minimum levels of vitamin A or carotene					
Vitamin A fed	20	+0.49 ±0.08	+0.2852 ±0.0369	0.10	77
Carotene fed	19	+0.49 ±0.11	+0.2911 ±0.0738	0.12	48
Both dietary groups	39	+0.50 ±0.05	+0.2846 ±0.0291	0.11	72

[a] Standard error

Only slight differences in these relationships existed between Guernseys and Holsteins fed the vitamin A depletion ration and between Holsteins fed minimum levels of vitamin A and those fed minimum levels of carotene (Table 1). However, between calves fed the vitamin A depletion ration and those fed minimum levels of vitamin A or carotene the differences were more apparent ($P < 0.02$ for the intercepts). This appeared to be of biological interest, since, for the calves fed the depletion ration, the blood plasma level of vitamin A was being maintained primarily from liver stores of vitamin A. For the calves fed minimum levels of vitamin A or carotene the opposite function might be considered;

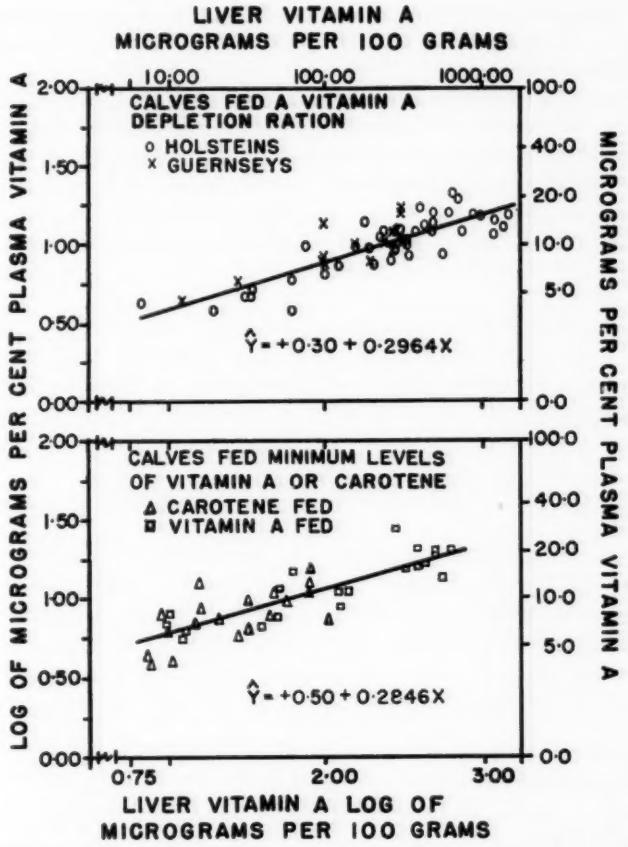

FIG. 1. Relationship of plasma vitamin A concentration to liver vitamin A concentration.

that is, the blood plasma vitamin A was being obtained via the intestinal wall and the liver stores of vitamin A from the blood plasma vitamin A. Thus it would be anticipated that for a given liver concentration of vitamin A, calves fed the depletion ration would have a lower blood concentration of vitamin A than calves fed levels of vitamin A or carotene sufficient to support constant blood and liver levels of vitamin A. The data support this expectation: very similar slopes but intercepts of +0.30 and +0.50 respectively for the depletion group and for the vitamin A and carotene fed group. If total liver stores are considered, it is necessary to recognize that the ratio of liver weight to body weight decreases as the calf becomes larger *(3)*. Therefore in small calves the total stores of vitamin A in the liver in relation to body weight at a given liver concentration of vitamin A are greater than in large calves. The similarity in slopes of the depletion group and of the vitamin A and carotene groups suggests

that the relationship between concentrations of vitamin A in the liver and in the blood may be independent of the size of the calf; however, this awaits experimental confirmation.

It was apparent from these data that plasma vitamin A concentrations when expressed as logarithms were directly related to liver vitamin A concentrations, also expressed as logarithms. The applicable limits for this relationship were for calves fed the vitamin A depletion ration 0.78 and 3.18 log γ concentration of vitamin A per 100 g. of liver (6 and 1,526γ of vitamin A per 100 g. of liver) and for calves fed minimum levels of vitamin A or carotene 0.76 and 2.79 log γ concentration of vitamin A per 100 g. of liver (6 and 620γ of vitamin A per 100 g. of liver).

To utilize this information to obtain the best estimate of the vitamin A concentration in the liver from the vitamin A concentration in the blood plasma, it was necessary to derive the regression of liver vitamin A concentration on plasma vitamin A concentration *(5)*. For the calves fed the vitamin A depletion ration this was:

1) $\hat{X} = -0.02 + 2.3724Y \pm 0.285$[2] in which $\hat{X}$ = expected liver vitamin A concentration expressed as the logarithm of micrograms of vitamin A per 100 g. of liver and Y = plasma vitamin A concentration expressed as the logarithm of micrograms of vitamin A per 100 ml. of plasma. Similarly, for the calves fed minimum levels of vitamin A or of carotene this relationship was:

2) $\hat{X} = -0.77 + 2.5336Y \pm 0.331$

The standard error *(5)* of $\hat{X}$ for a particular value of Y obtained for similar conditions under which the data for the derivation of equation 1 were collected is:

3) $$s_{x\text{-}\hat{x}} = 0.285 \sqrt{\left\{ 1 + \frac{1}{58} + \left[\frac{(Y-1.00)^2}{1.9123} \right] \right\}}$$

and for a value of $\hat{X}$ for a particular value of Y obtained under similar conditions under which the data for the derivation of equation 2 were collected is:

4) $$s_{x\text{-}\hat{x}} = 0.331 \sqrt{\left\{ 1 + \frac{1}{39} + \left[\frac{(Y-0.99)^2}{1.6346} \right] \right\}}$$

Applicable limits for the use of equation 1 were 0.56 and 1.33 log γ concen-

[2] Slight curvature was found upon introducing the quadratic term. However, since *(a)* this accounted for only a slight reduction in total variability about log micrograms of vitamin A per 100 g. of liver, 2.5%, and a reduction of 8.3% over that accounted for by the linear function; *(b)* the maximum value found for the quadratic relationship was 34γ% plasma vitamin A, a value well beyond the limits of the data studied; and *(c)* a linear function will describe adequately portions of a quadratic function in which there is only slight curvature, the situation described herein, the tendency for curvature was disregarded for purposes of this study.

tration of vitamin A per 100 ml. of blood plasma (3.6 and 21.4γ of vitamin A per 100 ml. of blood plasma) and for the use of equation 2, 0.58 and 1.41 log γ concentration of vitamin A per 100 ml. of blood plasma (3.8 and 25.8γ of vitamin A per 100 ml. of plasma). Both equations 1 and 2 should be of value in estimating the vitamin A storage of the calf within the limits cited. It is readily apparent that additional data beyond the upper limits are required before the relationship of plasma vitamin A and liver vitamin A concentrations can be described in its entirety for the dairy calf.

SUMMARY

Data on blood plasma vitamin A and liver vitamin A concentrations from 58 Guernsey and Holstein calves fed a vitamin A depletion ration and whose average age and body weight was 128 ± 19 days and 263 ± 72 lb., respectively, and from 39 Holstein calves fed minimum levels of vitamin A or carotene and whose average age and body weight were, respectively, 200 ± 9 days and 436 ± 48 lb. were explored as to possible relationship. When plasma vitamin A and liver vitamin A were expressed as logarithms, a positive linear relationship was found between these two variables. In calves fed the vitamin A depletion ration, the blood plasma vitamin A level was found to be less at a given concentration of vitamin A in the liver than in the calves fed minimum levels of vitamin A or carotene. Positive linear relationships of log liver vitamin A concentration on log plasma vitamin A concentration were derived for purposes of estimating liver stores of vitamin A in calves fed the vitamin A depletion ration and in calves fed minimum levels of vitamin A or carotene. These had applicable limits for prediction of approximately 3.6 and 25.8γ of vitamin A per 100 ml. of blood plasma.

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PEOPLE and EVENTS
in the Dairy Science World

Pioneers in the Dairy Industry

One important phase of the development of our industry has been the creation of the methods and equipment necessary for giving raw milk form utility. January, 1904, saw the beginning of a business enterprise, the Damrow Bros. Co. of Fond du Lac, Wis., that contributed much to this phase of the industry.

E. C. Damrow

After working for 4 years for A. J. DECKER, a manufacturer of cheese vats and the father of Professor JOHN DECKER, formerly a staff member of the dairy school at The Ohio State University, E. C. DAMROW at the age of 23 and his brother George started their own factory. In 1907 the company was incorporated to manufacture dairy equipment. The first year in business the company manufactured one 5,000-lb. noiseless water heating steam vat, which sold for $75. This type of vat, which was a radical departure from the conventional dry steam heating vat with a floating bottom, proved so popular that the following year the young company sold nine of their vats to their competitor's one. This firmly established Damrow Bros. as manufacturers of dairy equipment.

E. C. Damrow was born December 16, 1879, at Millersville, Wis. After 1 year of high school and at the age of 14 he got a job in a cheese factory as a helper and at 16 he obtained employment in a sheet metal works that manufactured cheese plant equipment. With this practical training and a correspondence course in sheet metal layout and drafting as a background, E. C. Damrow became head of one of the country's leading manufacturers of cheese plant equipment. The key to his success has been his willingness to work long hours, his honesty and integrity, and his creative imagination.

In the early days of the company young Damrow worked 12 to 14 hours per day. There were no company paid vacations or mid-morning and afternoon breaks for coffee in those days. Apparently such hours did not dull his imagination, for he began early to create new and improved items of equipment. When working as a boy with a cheese vat he learned by experience that cheese hoops were difficult to clean and sanitize. As a consequence, the Damrow cheese hoops were so designed that they could be more easily washed and sanitized. Another important contribution was the sanitary steam jet for moving whey, patented in 1915. The early curd mills either tore or shredded the curd apart or pressed the disc cutting knives against a solid wooden roller, causing considerable loss of butterfat. In 1923 the company brought out a new type of curd mill that completely sheared the curd into cubes; this not only was more sanitary but saved 1 to 2 lb. of butterfat for each 1,000 lb. of milk handled. Other equipment items developed by Mr. Damrow include the first traveling mechanical agitator suitable for longer vats so that two traveling paddles could be operated at one time singly or doubly; an improved cheese press designed to use less pressure at the beginning of the process and also to permit pressing several different sizes and shapes of cheese at the same time; a casein mill which later was used as a cheese grinder in the making of process cheese; and an improved straight-away and round can washer. As the business grew, new quarters had to be found; this resulted in the building of a new factory in 1916 with additions made in 1927, 1933, and 1945.

E. C. Damrow has not spent all his time in promoting his business. He has written a number of articles on cheese problems; during World War II he served on the War Production Board in the dairy industry division; he supervised the production of the film "Cheese Making," which has been shown in a number of universities. He is a member of numerous trade associations and has been active in the Lutheran church.

Possibly one of the finest contributions Mr. Damrow made was his aid in organizing and developing a summer camp at Mt. Morris, Wis., called the Retreat, for boys and girls of the city who otherwise would not have an opportunity to enjoy outdoor living.

In 1904 E. C. Damrow and Minnie Rautmann were married. They became the parents of three daughters and a son, Eugene, who lost his life in World War II. Mr. and Mrs. Damrow and their three daughters with their families spend their summers at the Retreat. In the winter the Damrows live at the Municipal Trailer Park, Tampa, Florida.

Mr. Damrow's hobbies are Skat and upholstering. He is chairman of the board for Damrow Bros. and is president of the First Federal Savings and Loan Co. of Fond du Lac. E. C. Damrow has contributed much to the dairy industry, to the people of the state in which he lives, and to his country. We are proud to have this opportunity to pay tribute to another of our great "Pioneers of the Dairy Industry."

Scientific Manpower at Midyear

A Report of the
Scientific Manpower Commission
1530 - P Street, N.W., Washington, D. C.

It took a technological war of world proportions to suggest that the problem of scientific and engineering manpower might profitably be studied, and the threat of another such war to initiate action. Inevitably, government agencies took the lead in analyzing those aspects of the problem that required immediate attention, but discontinuity of effort and uncertainty of purpose characterized official efforts and nullified any constructive or lasting result.

The Engineers Joint Council created the Engineering Manpower Commission in 1950, and in 1951 the American Chemical Society appointed a Committee on Manpower. The chemists were convinced from the start that most manpower problems are common to all fields of science and that a single agency acting in behalf of all the major groups would accomplish much more than separate committees working without coordination. This conviction was shared by the organizations in the other disciplines, and in 1953 their representatives joined forces to incorporate the SCIENTIFIC MANPOWER COMMISSION in the District of Columbia.

With a dozen different government departments and agencies and an equal number of private organizations working on one phase or another of the specialized manpower problem, the Commission's first task was to study all activities in the field and to establish working relations that would eliminate duplication of labor yet assure that a comprehensive job would be done.

In such highly specialized fields as the scientific disciplines, recruitment, training, and utilization are successive but integrated problems. Superimposed on the troublesome dilemma of an inadequate supply of scientists to meet civilian demands was their liability for military service. The treatment accorded graduate students from May, 1953, until June, 1954, became completely irrational, and Selective Service announced as a policy its intention to induct graduate assistants without regard to the status of their graduate work. Graduate schools promptly supplied the Commission with facts and figures on arbitrary and ill-timed inductions of students in mid-career, and the Commission's findings were placed before the Office of Defense Mobilization. Action was soon taken, and students and university departments will no longer be harassed by the uncertainties of badly timed and arbitrary reclassifications and inductions.

The Commission has also insisted on the adoption of more rational handling of occupational deferments, and, after a year in which it looked as if the policy was to draft all scientists without regard to their irreplaceability in critical occupations, the Selective Service System should soon be directed to adhere to the intent of the deferment provisions in the Selective Service law. In 1955 it will be necessary for Congress to pass new legislation, in which it is hoped basic principles will be spelled out in sufficient detail to place them beyond dispute, and in which the chaotic reservist situation will be put in order. The Commission has been assured that its views will be sought when the prospective bills come up for consideration.

There would be little point in all this activity on the proper utilization of scientists for national security if there were not a shortage—at least in certain fields. The register of scientific personnel that is being prepared for the National Science Foundation will provide a check on the available supply, and the Office of Education's annual survey of college and university registrations and of degrees granted is a reliable tally of the number of new scientists and engineers that are added to the manpower pool each year. Very little, however, has been done to determine the demand and its relation to the supply. Unwilling to accept unsupported generalizations about current shortages, the Scientific Manpower Commission has undertaken its own survey. A comprehensive study of industry's immediate requirements for scientists in each major field has been completed, and the results will soon be available. A comparable survey of demand for fiscal year 1954-55 in government agencies is in progress, and this will be followed by a study of requirements for teaching personnel during academic year 1954-55.

Although there is always a shortage of high-caliber scientists, programs of recruitment should bear some rational relationship to prospective demand, and the results of the SMC surveys should be guides for action in each field of science. In preparation for service in this vital facet of the manpower problem, the Commission has compiled a bibliography of literature on career guidance and has assembled a file of material in current use among student counselors.

Pamphlets, however, are no substitute for the inspiration that good teachers and good counselors can provide, and for this reason SMC has joined with the Engineering Manpower Commission to face the most acute shortage of all—namely, that of high school teachers of science. Working with the National Science

Teachers Association, the Future Scientists of America, the AAAS Cooperative Committee on the Teaching of Science and Mathematics, the Academy Conference of the AAAS, the Thomas Alva Edison Foundation, and the Educational Council of the National Association of Manufacturers, it is now seeking the cooperation of its sponsoring agencies and their affiliates in a nationwide effort to resuscitate science where most young men and women have their first introduction to it. The Commission is also anticipating the time—a scant four years ahead—when the colleges will feel the pinch that has just gripped the high schools.

Science and technology are abstract terms that acquire substance only through people—men and women with the right temperament and with minds disciplined by prolonged training. Like any resource, the supply is limited—though renewable. Yet we have known less about it than about the size of our coal reserves or of our wheat crop. In fifteen months of operation, the Scientific Manpower Commission has begun to perceive the many facets of the manpower problem and to see how some of them may be resolved. The degree of success achieved will be directly proportional to the support and cooperation given not only by its sponsors, but by scientists themselves. It is the profession's problems, rather than the Commission's, that must be faced.

Hibbs Succeeds Hansen at Idaho

R. A. HIBBS, for the past 4 years in charge of the quality control program of the Inland Empire Dairymen's Assoc., has joined the staff of the Department of Dairy Husbandry at the Univ. of Idaho. He succeeds H. C. HANSEN of the dairy manufacturing section, who retired July 1, after completing 29 years of service in dairying.

With headquarters in Spokane, Hibbs has been engaged in commercial work since he received his doctor's degree from the State College of Washington in 1951. He received his undergraduate training at the Univ. of Florida.

Although retired from the dairy field, Dr. Hansen will remain active in civic and community affairs, having been appointed Moscow postmaster.

C. W. England Establishes Consulting Service

C. W. ENGLAND, former Technical Director of the C. Y. Stephens Dairy Industries, Washington, D. C., has established the C. W. England Laboratories, 1615 First Street, S.W., Washington, D. C. Dr. England graduated from the Univ. of Maryland in 1923 and received his graduate training at Cornell Univ. For several years he was in charge of dairy manufactures at the Univ. of Maryland.

Tennessee Events

A milk sanitation seminar was held at the Univ. of Tennessee November 16-18 for the purpose of demonstrating high-temperature, short-time pasteurization tests. A short course to train technicians for artificial insemination was held Oct. 18-23. The annual meeting of the Univ. of Tennessee Dairy Committee was held November 12. This group includes dairy teaching, research, and extension workers located throughout the state.

R. A. MONROE, who has been carrying on research with isotopes for the Atomic Energy Commission at Oak Ridge, is now listed on the research staff of the Univ. Dairy Department.

Nelson Joins Dairyland Food Labs.

J. H. NELSON, formerly in the Dairy and Food Industries Department at the Univ. of Wisconsin, has accepted the position of Director of Research for the Dairyland Food Laboratories, Inc., of Waukesha, Wis.

Irving C. Reynolds Receives the Sixth Ohio State University Award of Merit

IRVING C. REYNOLDS, president of the Franklin Ice Cream Company, Toledo, Ohio, was presented with the 1954 Dairy Technology Award of Merit for his outstanding contributions as a citizen, a successful businessman, and as an outstanding national and international leader in the dairy field. L. L. RUMMELL, dean of the College of Agriculture, presented this award at the annual Dairy Technology Homecoming celebration on October 23.

Mr. Reynolds founded the Franklin Ice Cream Company in 1921 and has been its president since it was incorporated in 1926. Under his guidance this company has prospered and expanded in both Michigan and Ohio. This organization has been a pioneer in retail ice cream store operation, and its progressive merchandising methods have set the pattern for many to follow. In 1933 he was one of the organizers and first president of the National Assoc. of Retail Ice Cream Manufacturers.

He has served his country in two World Wars: in World War I as a foot soldier, in World War II as Chief of Procurement for Perishable Foods. For the latter, he received the Exceptional Service Award from the War Department.

In 1947, he represented the United States in visiting the Far East to arrange for milk supplies for the armed forces and civilians. He recommended the establishment of seven dairy plants, all of which were completed. In 1951, he studied the dairy industry in northern Europe and early this year as a special representative of the U. S. Department of Agriculture he visited nations of the Far East and Near East in search of market outlets for milk and milk products. One year ago he represented the

Dairy Industries Society International as a dairy consultant to the government of Puerto Rico.

D. H. Jacobsen Named ADA Research Director

D. H. JACOBSEN has joined the staff of ADA as research director succeeding R. E. FROST, who has moved into the organization's membership department.

For the past 10 years Jacobsen has been technical advisor in the supply and merchandising department of Cherry-Burrell, Inc., Chicago. Prior to that he was quality control supervisor for the Prairie Farms Creameries, Chicago.

The new research director received his B.S. degree from South Dakota State College, his M.S. degree from Washington State, and his doctor's degree from Iowa State College. He taught for 10 years in the Dairy Manufacturing Department of South Dakota State College and also engaged in research work during that period. He will supervise the nutrition, market and economic research program being conducted by ADA.

Staff Changes in Penn State Extension

Several changes in staff personnel have taken place in the extension section at The Pennsylvania State Univ. J. S. TAYLOR has been named chairman of the Dairy Science Extension Section, succeeding R. H. OLMSTEAD, who retired July 1. Professor Taylor has been a member of the extension staff for nine years. His activities in the Penn State extension program have included special organizational work in the artificial breeding program in Pennsylvania and educational phases of the Bang's control program. His future work, in addition to administrative details of the section, will follow similar lines.

H. W. THOELE joined the dairy science extension staff as a dairy specialist Nov. 1, 1954. He is a graduate of the Univ. of Minnesota and also holds M.S. and Ph.D. degrees from that institution. He will work as an extension specialist in the field of dairy cattle breeding.

R. S. ADAMS also joined the extension staff on November 1. He was graduated from the Univ. of Maine in 1950. Dr. Adams did his graduate work at the Univ. of Minnesota. He will work in the areas of dairy cattle feeding and management.

Huston Joins Staff at Kansas State

K. A. HUSTON has accepted a position on the dairy husbandry staff at Kansas State College. Dr. Huston will be in charge of instruction and research in dairy cattle genetics. He completed his advanced study at the Univ. of Wisconsin and has spent the past 2 years at the Virginia Polytechnic Institute as a member of the dairy extension staff.

Kosikowski to Study in France

F. V. KOSIKOWSKI, professor of dairy industry at Cornell Univ., has been selected for a Fulbright award as Research Scholar in France, beginning the second semester of the present academic year. He will collaborate on dairy bacteriological and chemical research problems with DR. GERMAIN MOCQUOT, director of the Central Laboratory of Microbiology and Milk Research at the National Agricultural Research Institute in Paris. Dr. Mocquot, one of France's youngest research directors, recently has become the corecipient of the Gorini prize given by the Italian government for outstanding work on the nature of the natural bacterial inhibiting factor of milk. Dr. Kosikowski, with his wife and daughter, will leave for France by steamship some time in the latter part of January. His address will be the U. S. Educational Commission for France, 9 Rue Chardin, Paris 16.

West Virginia News

The W. Va. Dairy Products Assoc. has announced the recipients of three $250 scholarships in dairy manufacturing at W. Va. Univ. Awards are based on scholarship during the freshman year and are limited to dairy manufacturing majors. This year's scholarship winners are GARETH MATLICK, Newburg; A. ZANE STILES, St. George; and ORMAN G. TYREE, Marlinton.

ERNESTO KASDORF, Buenos Aires, Argentina, a graduate of the Facultad de Agronomia y Veterinaria Buenos Aires, has been granted a fellowship in dairy manufacturing at W. V. U. Prior to coming to W. Va. he spent 6 months in Europe studying dairy plant operations, including 3 months at the German Dairy Research Institute at Kiel. His father is the owner of a large milk plant in Buenos Aires. Mr. Kasdorf plans his Master's thesis on yoghurt, which is an important dairy product in Argentina.

Frayer Retires

J. M. FRAYER, dairy bacteriologist, retired July 1 after 27 years service with the Vermont Agricultural Experiment Station. His varied research work has contributed valuable information on factors relating to production of

quality milk and laboratory diagnosis of bovine mastitis. He has written many articles and bulletins on these fields of work.

News from Montana State College

The Dairy Industry Department has moved into a new addition to the Agriculture Building, which has just been completed. The new quarters consist of private offices for all staff members; testing, research, and production laboratories; separate processing rooms for butter and cheese, milk, cultured milks, and ice cream. An auditorium is available which is equipped for visual education.

The 19th annual Dairy Industry Short Course will be held Nov. 29 through Dec. 1. The first two days will be devoted to dairy manufacturing and dairy technology and the last day to dairy production problems. G. A. RICHARDSON, Oregon State College; N. S. GOLDING, State College of Washington; R. S. GUTHRIE, De Laval Separator Co.; D. C. LIGHTNER, Creamery Package Co.; and N. C. ANGEVINE, Meyer Blanke Co., will be guest speakers. A dairy products show consisting of entries of butter, cheese, and ice cream will be held in connection with the course.

The Montana Dairy Industries Assoc. has granted a $150 scholarship to the Dairy Industry Department. This scholarship is to be known as the "Doctor J. A. Nelson Scholarship Fund of the Montana Dairy Industries Association," and will be used for promoting increased enrollment in dairy manufacturing.

Arizona Increases Size of Dairy Farm

The Univ. of Arizona has purchased a farm for use by the Department of Dairy Husbandry. It includes about forty acres of irrigated land and additional space for buildings and feed lots.

The Annual Dairy Industry Conference at the Univ. of Arizona will be held January 14, 1955. The program will deal with dairy plant efficiency. Guest speakers will include C. A. IVERSON from Iowa State College.

New Jersey to Give Scholarships

A new 4-year scholarship worth $1,000 has been established at Rutgers Univ. for young people interested in careers in dairy administration, DR. L. W. JONES, president, has announced.

Named the John C. Welsh Memorial Scholarship in honor of the late president of Welsh Farms, Inc., the new award will be given each year to an entering freshman enrolled in the new Rutgers course in dairy administration. The new program will provide training in business methods and a background in liberal arts, in addition to a knowledge of dairy technology.

Scholarship winners will be selected each year from among secondary school graduates with preference given to New Jersey residents. The scholarships will provide $250 each year for the full 4 years of the college course. A new winner will be selected each year until, by the start of the 1958 school year, there will be four of these award holders in the dairy administration program at Rutgers.

British Dairy Scientist to Visit U. S.

In late October A. W. MARSDEN, director of the Commonwealth Bureau of Dairy Science, Shinfield, Reading, left England to undertake a 4-month tour of some dairying centers in Pakistan, India, Australia, New Zealand, and the United States. He will also pay short visits to F.A.O. in Rome and to Singapore and the Fiji Islands. Mr. Marsden was appointed director of the Bureau in 1950 after being head of the Chemistry Department at Seale-Hayne Agricultural College and a lecturer at the Imperial College, London.

Collegiate Students' International Contest in Judging Dairy Products

Teams from 26 U. S. land grant colleges and universities participated in the 20th contest, sponsored annually by American Dairy Science Assoc. and Dairy Industries Supply Assoc., Inc., on October 25 in Atlantic City's Convention Hall. Following is a list of those who won high standings in the contest:

ALL PRODUCTS

Individuals

1. JOHN R. DOYLE, University of Minnesota
2. DELBERT MERRITT, University of Nebraska
3. WILLIAM H. BERGMAN, Kansas State College
4. CHARLES WALES, Texas Technological College
5. DAVID E. MIZER, Ohio State University
6. EDMUND JANUS, Mississippi State College
7. RAYMOND A. JAWORSKI, University of Connecticut
8. RICHARD B. MARTIN, University of Tennessee
9. WALTER W. JOHNSON, University of Tennessee
10. ROBERT E. ADAMS, West Virginia University

Teams

1. Kansas State College
2. Mississippi State College
3. Texas Technological College
4. University of Tennessee
5. University of Minnesota

6. University of Connecticut
7. Pennsylvania State University
8. Cornell University
9. Michigan State College
10. West Virginia University

BUTTER

Individuals

1. Eugene Scott, Michigan State College	6.00
2. Ray A. Jaworski, University of Connecticut	6.67
3. John A. Willemsen, Washington State College	7.00 (F1:5.5)
4. B. E. Bevier, Michigan State College	7.00 (F1:7.0)
5. W. F. Stoll, Iowa State College	8.50

Teams

1. Iowa State College	29.0
2. Michigan State College	29.67
3. University of Connecticut	32.84
4. Mississippi State College	34.17
5. University of Vermont	42.00

MILK

Individuals

1. Alva E. Williams, Jr., North Carolina State College	22.75
2. Dixie M. Keppel, Michigan State College	26.00
3. Carol M. Yarborough, North Carolina State College	26.34
4. Billy G. Freeman, Alabama Polytechnic Institute	27.50
5. Harold Schultz, South Dakota State College	29.00

Teams

1. North Carolina State College	81.26
2. Virginia Polytechnic Institute	103.75
3. University of Tennessee	109.25
4. Kansas State College	112.35
5. University of Maryland	113.00

ICE CREAM

Individuals

1. Charles Wales, Texas Technological College	26.50
2. Robert P. Silva, University of Connecticut	27.17
3. William H. Bergman, Kansas State College	27.75
4. Ralph Scharr, Cornell University	28.17
5. Eugene Scott, Michigan State College	28.17

Teams

1. Kansas State College	89.25
2. University of Connecticut	95.02
3. Pennsylvania State University	99.01
4. Cornell University	99.56
5. Iowa State College	99.67

CHEESE

Individuals

1. William H. Bergman, Kansas State College	21.33
2. Stanley Pollock, Cornell University	25.33
3. Walter W. Johnson, University of Tennessee	25.67
4. David E. Mizer, Ohio State University	26.47
5. Robert E. Adams, West Virginia University	26.47

Teams

1. University of Tennessee	87.52
2. Clemson Agricultural College	91.28
3. Mississippi State College	92.18
4. Kansas State College	96.26
5. Oklahoma A & M College	96.31

C. J. Babcock, of the Foreign Agricultural Service, USDA, was contest superintendent. He was assisted by D. R. Strobel, also of FAS. G. M. Trout, Michigan State College, headed the American Dairy Science Assoc. contest committee and Charles Weinreich, Cherry-Burrell Corp., Chicago, had charge of the corresponding committee of Dairy Industries Supply Assoc.

Product judges were R. Whitaker, National Dairy Research Lab., milk; N. E. Fabricius, Ladysmith (Wis.) Milk Producers Cooperative Assoc., butter; H. L. Wilson, Kraft Foods Co., cheese; and J. Hoffman Erb, The Borden Co., ice cream.

Winners of Student Judging Contest at International Dairy Show

The Illinois cattle judging team and the Iowa State products judging team were named champions at the International Dairy Show held at Chicago on October 9. The Illinois cattle judging team was one of 15 participating in the contest. Earl Kingman, a member of the Illinois team, was the high scoring individual.

In the products judging, the Iowa State team, besides winning first place in all products, won the butter, milk, and ice cream cups. The Ohio State team took first place in judging cheese. The high individual in the products judging was Jack Pollei of Iowa State.

National Intercollegiate Dairy Cattle Judging Contest Winners

The dairy cattle judging contest held at Waterloo, Iowa, October 4 was participated in by 29 teams. The ten high teams were:

Placing	*Team*	*Score*
1st	Arkansas	1,919
2nd	Iowa State	1,903
3rd	Illinois	1,892
4th	Kansas	1,885

4th Tie	Penn State	1,885
6th	North Carolina	1,876
7th	Oklahoma	1,875
8th	V.P.I.	1,863
9th	Kentucky	1,849
10th	Cornell	1,840

The high individual in the contest was ALLAN HEATH of the Kansas team. JOHN HARRIS of Penn State and R. J. CURTIS of the Ontario Agr. College tied for second place.

The high teams in judging the different breeds and their scores were:

AYRSHIRE

Teams

1. Oklahoma A & M	415
2. Arkansas	404
3. Texas A & M	395
4. Cornell	394
5. Kansas	390
5. Kentucky	390

Individuals

1. GEORGE LOUPEE, Iowa	146
2. ALLAN TURNER, Massachusetts	145
3. HERBERT REBHAN, Kentucky	141
3. MILTON WELLS, Oklahoma	141
3. L. M. BRAZIEL, Texas A & M	141

BROWN SWISS

Teams

1. Cornell	419
2. Washington State	408
3. Penn State	406
4. Iowa State	402
5. South Dakota	399

Individuals

1. JIMMIE VERDIN, Clemson	143
2. DON BAY, Cornell	142
2. DON PLOTE, Illinois	142
2. DON FUNK, Iowa State	142
5. RICHARD HARRIS, North Carolina	140
5. WILLIAM BURNS, Purdue	140
5. CARL MANSPERGER, Washington State	140

GUERNSEY

Teams

1. Arkansas	413
2. Ontario Agricultural College	411
3. Kansas	386
4. Oklahoma	384
5. Colorado	378
5. Texas Tech.	378

Individuals

1. BURRELL SMITTLE, Arkansas	144
2. DARYL PULTS, Kansas	143
3. JOHN WOESTE, Kentucky	141
4. GORDON FORD, Arkansas	140
5. THOMAS COYNE, Cornell	139

HOLSTEIN

Teams

1. Washington State	402
2. Maryland	401
2. Purdue	401
4. Minnesota	399
4. North Carolina	399
4. Ontario Agricultural College	399

Individuals

1. CHARLES BARTH, Washington State	147
2. BRUCE LARSON, Minnesota	143
3. WILLIAM BERGMAN, Kansas	140
3. ROGER RICHARDS, Nebraska	140
3. DON RICHARDSON, Ohio State	140

JERSEY

Teams

1. Michigan	428
2. Illinois	409
3. Arkansas	405
4. Penn State	394
5. Washington State	390

Individuals

1. RICHARD LAING, Michigan	147
2. JOHN HARRIS, Penn State	146
3. HAROLD HODGES, Oklahoma	145
4. GORDON FORD, Arkansas	144
4. JOHN ADDINGTON, Michigan	144
4. JACOB GUFFEY, Penn State	144

New Products, Ideas, and Services Highlight Displays at 19th Dairy Industries Show in Atlantic City, October 25-30

Among the top new developments that enabled the dairy industry's processors of milk and manufacturers of dairy products to "Cross Tomorrow's Threshold"—the appropriate slogan for the 1954 Exposition—were these that follow under the heading of various commodity or service fields.

Dairy Plant Equipment

Considerable interest was shown in a machine which pasteurizes ice cream mix at 260° F., which can heat up to 300° F. on a continuous basis to pasteurize fluid dairy products without imparting a cooked or caramelized flavor.

Also on display was a machine which produces standard quarter-pound prints already sliced into patties or chips that don't stick together. An automatic butter moulding and wrapping machine moulded soft butter directly from the churn.

Cheesemakers found an important new machine in a new stainless steel mechanized agitator for Cheddar cheese. The fluid milk operator for the small or medium size dairies found fillers especially made for them and having an automatic detector, which minimizes milk waste.

A new clamp designed for cleaned-in-place pipeline operation portrayed a new concept of fitting procedure—it offers greater speed and ease of installation, savings in original investment cost, and longer, trouble-free, life. It can be used in conjunction with standard 3-A Sanitary Standard fittings by use of a simple adapter.

A fully automatic rotary machine for fast, economical production of ice cream bars and twin ice cream bars was shown. This machine, without auxiliary equipment, fills molds, freezes, inserts sticks, defrosts, dips into chocolate and bags . . . in one continuous operation.

Containers and Closures

Special attention was focused on the new gallon and half-gallon milk jugs which have attracted so much consumer interest recently.

The small-company dairyman found plastic paper milk cartons designed for his use along with proper sized filling equipment.

All types of materials—glass, parchment, aluminum, tin, stainless steel, dairy metal, etc. —were displayed in this field. Aluminum foil packaging, for example, showed its latest advances, with top interest shown in the new heat sealing rotogravure printed aluminum foil ice cream carton overwraps and ice cream sandwich overwraps.

Delivery Equipment

A highlight in this field was the new plastic milk tank in 1,500-gallon size for farm pick-up and 4,000-gallon size for over-the-road transport. Also a new refrigerated truck body which utilizes a hydraulically operated constant-temperature refrigeration system that runs off the truck engine and which requires no major chassis revision. A refrigerated retail milk delivery body equipped for 24-hour refrigeration was on display.

An important subsidiary to this delivery field was an automatic composite butterfat sampler for checking butterfat content during the loading and unloading operations of tank-cars and trucks. It works only when the milk pump is operating.

Sanitation Chemicals Field

There was a demonstration of C-I-P cleaning utilizing a new type of material referred to in the industry as a "chlorinated cleaner" which produces enhanced cleaning ability when built into the formula. This same demonstration utilized a Geiger counter which, through the use of radioactive tagged soils, showed visitors that without the use of proper cleaning agents, a film of dirt invisible to the human eye still remains.

An acid milkstone remover with wetting agent added for quicker and better cleaning was demonstrated to show its effectiveness for use in C-I-P, high-temperature short-time, and milking machines.

There was a new liquid cleaner, containing a neutral wetting agent that is harmless to metals, milking machine rubbers, or hands. Its composition makes possible quick and effective dirt removal.

A handy-type sterilizing gun for properly sterilizing farm pick-up, milk storage, and transportation tanks was also displayed.

Point of Sale Equipment

A recent dairy industrial innovation is the outdoor milk vendor, which enables the housewife to get milk for her family by serving herself at the road-side machines. One vendor of this type on display was a high-volume 24-hour service machine designed to sell paper cartons of milk by quarts, two quarts, one-half gallon or two one-half gallons through a single operation of the coin mechanism.

Miscellaneous Commodities and Services

A scale model gave visitors a look at tomorrow's dairy plant—this plant portrayed the move toward simplification of effort and increased efficiency in the production of top quality milk and dairy products.

Another display showed a unique, inexpensive gadget to check pressure-sealed systems for liquid-level, gas-liquid ratio, cleanliness of liquid, and operation of sealed valves. This field also contained fly-repelling doors and information on manufacturing certain specialty products.

Special interest was shown in the new advertising and promotion techniques—with emphasis on the now accelerated supermarket promotion. The dairy trade press showed off its new format designs and informative departmental features—the latest information on the increasing technological changes within the industry. Over 25,000 people attended the exposition, which is held every other year.

Completed Theses

M.S. Degree:

G. I. PRITCHARD—Antibiotics in the nutrition of young dairy calves. Univ. of Vermont.

L. H. BUTTON—Comparative nutrition values of low and medium dry matter grass silages for growth of dairy calves. Univ. of Vermont.

E. P. MERRILL—A bacteriological study of retail ice cream dipping operations with special emphasis on the coliform group. Univ. of Vermont.

R. R. HEDRICK—A flavor survey of the milk produced by the Montana State College dairy herd. Montana State College.

Ph.D. Degree:

E. P. SMITH—A study of the estrogen content of cows' urine as related to estrus, pregnancy, and parturition. State College of Washington.

GERALD SILVERMAN—The qualitative and quantitative determination of certain nitrogenous compounds in Cheddar cheese made from raw and pasteurized milk. Cornell Univ.

LETTERS TO THE EDITOR

To the Members of the American Dairy Science Association:

As the President of the A.D.S.A., I would like to make use of the medium of the Journal to convey to the members the effect of some of actions of your Executive Board taken at the annual meeting at Penn State University last June. It was voted to increase membership dues from $8 to $10 per year. You will note that this action places the Board in the category of "rugged individualists," as defined in Walter Price's presidential address at Penn State. His address should be preferred reading for those interested in the welfare of our Association and can be found in the August, 1954, issue.

This increase in membership dues will help in a large way to take care of increased costs in publishing our Journal, in which we invest about 77% of our budget each year. Publishing this fine Journal is by far our most important activity.

I am sure all of you are aware of the new look our Journal has taken since Paul Tracy has become editor. We now have several new sections, namely, People and Events in the Dairy Science World, Letters to the Editor, and Our Industry Today, where brief reviews of current topics are published. The possibility of having a student affiliate page is now under consideration.

To me these additions to the Journal are a natural result of the growth and broadening interests of our Association. We cannot afford to remain static, which is the easiest state of existence. Instead we must reflect the dynamic state of our industry. According to the survey conducted last year by the Journal Management Committee, the majority of the members like these new features in our Journal (see page 902, the July, 1954 issue). When I receive my Journal I unknowingly turn to these sections first, I suppose to find out what is going on in the dairy industry. I am sure many of you do the same. You can help make these features even more interesting by furnishing the editor with current information from your area.

This broader outlook of our Journal will attract a wider spectrum of people in the dairy industry, which should result in new members joining our Association, especially those in industry. Those of you who belong to other scientific organizations know that a $10 membership fee is about average, with some considerably higher.

Another action taken by the board was to increase the charge for reprints as reported on page 1007 of the August, 1954, issue. This matter was discussed by the board for a couple of years before final action was taken. It was felt that the Experiment Stations and the Government were receiving a "free ride" in getting their research results published at the expense of our Association. It is our belief that research organizations should help in defraying a part of the publishing costs since they do not have to issue bulletins to report research results. Even with the increased cost, reprints are still one-third to one-half cheaper than the cost of publication in bulletin form. Furthermore, most scientists like to have their papers appear in the same publication as the work of their colleagues.

It was the hope of the "rugged individualists" on the board that these actions will balance our budget for the coming year. Although our Association is in excellent financial state because of the existence of a healthy reserve, we can not continue for long spending $3000 more each year than we receive.

I feel that these actions of the board are in the best interests of the Association and believe that they will be acceptable by the great majority of our members.

L. A. MOORE
President
American Dairy Science Association

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Developments and Trends in Dairy Farm Building Design and Construction

THAYER CLEAVER

Agricultural Research Service, USDA, University of Illinois, Urbana

Early dairy structures, many of which were crude and skimpy, had some good points. These structures, which served as windbreaks and shelters from storms, were made with local materials such as stones and poles and had a brush or straw roof. Such shelters were small because only a few cows were kept by most farm families. But the cattle didn't necessarily suffer from lack of adequate shelter. If they got skinny and produced little or no milk during the winter months it was most likely due to a lack of good feed.

This was the type of structure many of our grandfathers had. After a few years they built pole frame structures of durable native materials similar to those of today. Then they piled straw around the east, north, and west sides and over the top because straw was more plentiful and cheaper than boards. They built a small enclosed shelter large enough to hold from four to six cows, where they were tied with a rope and fed grain while they were hand milked. Actually these pioneers had a loose-housing system, no different in principle from what we have today.

But most of the early dairymen who increased the size of their herds drifted away from the loose-housing method. They built larger and tighter barns, many of which were the general purpose type which housed several kinds of livestock with only a part of the building used for dairy cattle. This type of structure to a large extent has been gradually replaced by the stall barn which houses only dairy cattle, though many of the older barns in the midwestern states — both dairy and general purpose types — are still in fairly good condition. One of the problems facing dairymen today is how to remodel or convert these old buildings into modern, flexible dairy layouts. It is advisable to plan new buildings with sufficient flexibility that they can be easily converted to other uses if the dairy enterprise is discontinued.

It is also important to build structures that are less costly than many that are in use today. Because of their fixed character and the effect which their design and arrangements have on efficient production, dairy structures are of more importance in comparison with other dairy investments than may be indicated by their relative cost. Although dairy building costs have not risen as much as the cost of dairy cows, labor, feed grains, and hay since World War II, the total dairy building investment amounts to about one-half of the total dairy investment in some midwest areas. The table below shows the farm and dairy investments in the Chicago and St. Louis areas.

TABLE 1

Farm and dairy investments, 1947, for 200 farms in Chicago area and 150 in St. Louis area

Item	Chicago area		St. Louis area		Both areas	
	Percent of total farm investment	Percent of dairy investment	Percent of total farm investment	Percent of dairy investment	Percent of total farm investment	Percent of dairy investment
Service buildings	16	46	20	53	17	49
Land and land improvements	62	([a])	57	([a])	60	([a])
Power and machinery [b]	5	3	8	4	6	3
Productive livestock	10	30	7	23	9	27
Feed	7	21	8	20	8	21
Total	100	100	100	100	100	100

[a] Use of land was charged to the dairy enterprise in the form of feed costs for all farm-grown feeds utilized.

[b] Includes investment in horses and mules. This item covers only dairy equipment when referring to the dairy enterprise.

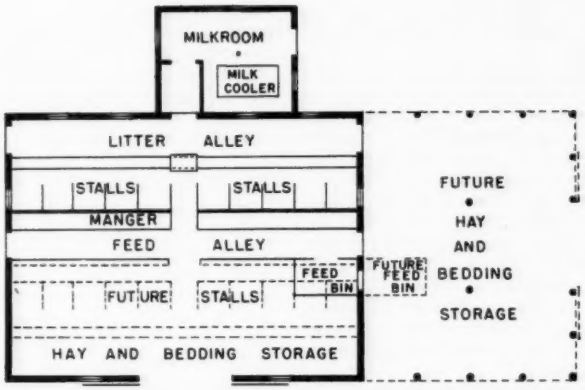


FIG. 1. A low cost, expansible, one-story stall barn. Although the plan shows 10 stalls initially it is also suitable for 12, 14, and 16 stalls initially with expansion to 20, 24, 28, and 32 stalls, respectively. Future expansion of stalls and feed storages is shown with dotted lines. The future feed storage area can be low cost, pole frame type of construction with paved floor optional.

How Can Stall Barns Be Improved?

Much has been done since World War II to reduce dairy building costs and to improve the serviceability of the stall barn. If the structure is limited to what is essential for production of quality milk, it can cost less than many of those that have been built in the past. Figure 1 illustrates a one-story barn which has a number of desirable features: *(a)* it will cost less to build than a two-story barn; *(b)* it has an efficient arrangement of work areas for the feeding and milking operations; *(c)* it is more flexible, that is, it can be more easily expanded and with less cost than a two-story barn, and *(d)* bedding, hay, and concentrates can be stored and fed more easily and economically than in most two-story barns, mainly because storage is at ground level. Silage can be stored in an upright silo at the left end of the feed alley or

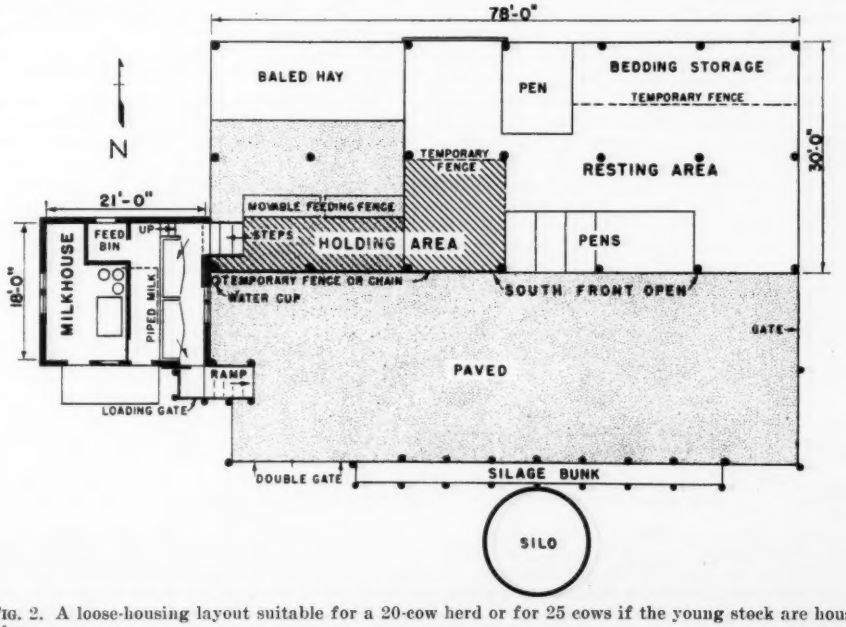


FIG. 2. A loose-housing layout suitable for a 20-cow herd or for 25 cows if the young stock are housed elsewhere.

in a horizontal silo located along one side of the paved lot from which it is fed into fence bunks or is self-fed.

Many of the older two-story barns have lofts which were designed for loose hay and many of those built more recently have sufficient capacity to store loose hay, though baled hay or chopped hay may be used. Such lofts may have twice the volume they need. Some unnecessarily large lofts have been built because the dairyman thought they looked better. Such structures are unnecessarily expensive, and they add to the labor and expense of storing and handling feeds. Dairymen have also learned that the feeding of silage or larger volumes of silage than were formerly thought practicable has reduced the amount of hay needed and hence the hay storage space needed.

Frequently, the amount of available labor for the daily chores limits the size of the dairy herd. When the labor supply is suddenly reduced and satisfactory hired labor is not available, the only alternatives frequently are to reduce the size of the herd to the number the farm family can care for; discontinue the dairy enterprise, or change to a system which will require less time, travel, and labor. The milking operations may be the labor bottleneck. In such cases the addition of an elevated stall milking room — one of the types shown in Figure 5 — will make it possible to care for the dairy herd with less labor.

Many dairymen have found that combining a stall barn with a loose-housing system means that less time and effort are needed for doing the daily chores. For example, they may use the stall barn only for milking, during which cows are fed their concentrates. Hay and silage are fed in the open paved lot or in an open front shelter, and the cattle have a separate resting area which is bedded. As soon as cows are milked they are returned to the feeding-resting shelters outside. Bedding is not used in the milking barn, comparatively little manure accumulates there, and the cleaning of the milking barn is thus simplified. A power scoop with a blade is used to clean the feeding and resting areas. This is actually a conversion to a loose-housing system.

Will It Pay to Convert to Loose-Housing?

The loose-housing system is a flexible arrangement of buildings and open lots developed for efficient milking and management of dairy herds. In this system the cows are kept in an open building, lot, or pasture and are not confined in stalls except while being milked. In contrast, the familiar stall barn has a stall for each cow where they are confined during cold weather except for short exercise periods.

Cows handled under the loose-housing system do not suffer from cold because in winter they grow thick coats of hair and have shelter from wind, rain, and snow. During the winter in cold climates manure and bedding are allowed to accumulate in a resting area to form a soft bed on which the cows like to lie. The manure pack generates some heat. If well managed, cows keep as clean or cleaner than in a stall barn and have less trouble from stiff hocks and swollen knees. In case of fire there is little danger of losing animals, since they are not confined in a building.

The cows are milked in a separate room or building that usually accommodates only part of the herd at one time. Cows enter singly or in groups and are released immediately after milking. An expanding herd can be accommodated by milking more cows in a stall and, if necessary, by using a larger milk cooler.

Hay and silage can be partly or completely self-fed, and the use of tractor manure loaders and power scoops will eliminate hand labor in

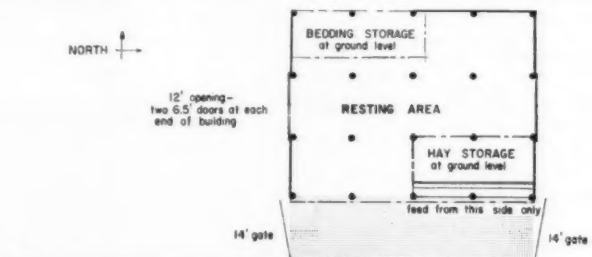


FIG. 3. Only 3 to 6 inches of feeding space per cow are needed in this silage self-feeding arrangement. The movable feeding fence can be placed on the floor or with a bunker silo supported from a cross pole on the walls. If not self-fed, silage can be fed in limited amounts in the fence bunk. Hay is fed on the open side of the barn along the hay storage area. This helps prevent unnecessary animal traffic in the resting area. The wide gates and doors provide a drive-through for convenient storing of hay and bedding. This layout is suitable for both dairy and beef cattle.

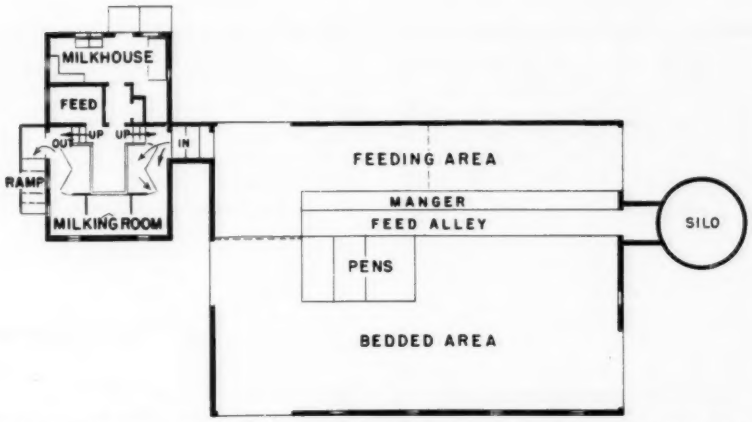


FIG. 4. A general purpose barn remodeled for loose housing. Unnecessary partitions and inner walls have been removed and doors widened to accommodate power machinery. The milking plant with 3 elevated stalls is new.

cleaning the feeding and resting areas and open lot.

The loose-housing system has some disadvantages. Men caring for the animals are more exposed to weather than in a stall barn. If the resting and feeding areas are not definitely separated, twice as much bedding may be required to keep cows clean as in a stall barn. However, if the system is well arranged and managed, little more, and sometimes much less, bedding is required than in a stall barn.

The Parts of a Loose-Housing System

A complete loose-housing layout has *(a)* a farm milking plant which includes a milking room or barn, a milkroom or milkhouse, and usually a concentrate feed bin; *(b)* roughage and concentrate feeding facilities for cows and young stock; *(c)* storage space for hay, silage, and bedding; *(d)* resting areas for adult and young stock; *(e)* maternity, hospital, and calf pens; and *(f)* an open lot, which should be at least partially paved.

In the mild climates of the South and Southwest, where cows are on pasture most of the year, building requirements are greatly simplified and only the farm milking plant with a holding pen may be needed.

Figure 2 illustrates the essential parts of a loose-housing system. Many different arrangements are possible, but the important thing is to keep the structures, including lots and fences, simple, serviceable, and economical. An efficient arrangement is also convenient and encourages good management, and most of the drudgery and heavy labor are eliminated.

The layout in Figure 2 is planned for a herd of 20 to 25 cows, but the two elevated milking stalls are suitable for herds as small as 8 to 10 cows. For herds much larger than 25 cows the feeding-bedding barn would be lengthened by one or more pole frame sections and more milking stalls would be added. An upright silo is indicated for open lot feeding of silage but a horizontal silo such as a trench, stack, or combination trench-stack (commonly called a "bunker" silo) may be located on a convenient side of the open lot for limited feeding of silage in fence bunks or self-feeding direct from the silo. Hay also can be fed from a simple roofed shelter in the open lot which has hay feeding mangers on two or more sides.

The feeding of hay and silage in the open lot, or self-feeding, has proven very satisfactory in most states and there is increasing evidence that it is satisfactory even in the northernmost states where severe winter weather occurs. Although pole frame type of construction is indicated in Figures 2 and 3, other types of construction may be even better. For example, a trussed roof, which would cost only a little more, would eliminate the interior poles and give a freer working space for power equipment. Figure 3 shows a suggested layout which is equally suitable for beef cattle. For dairy cattle the addition of a milking arrangement such as is shown in Figure 2 is all that needs to be added.

Old barns usually can be converted more economically and satisfactorily to a loose-housing system than to an improved stall barn. See Figure 4. Frequently all that is needed is the addition of a small farm milking plant with two to five elevated stalls, the removal of all unnecessary partitions and obstructions in the old barn, and making some of the doors wider for power equipment. Sometimes dairy stalls in the old barn can continue to serve satisfactorily for hay or silage feeding.

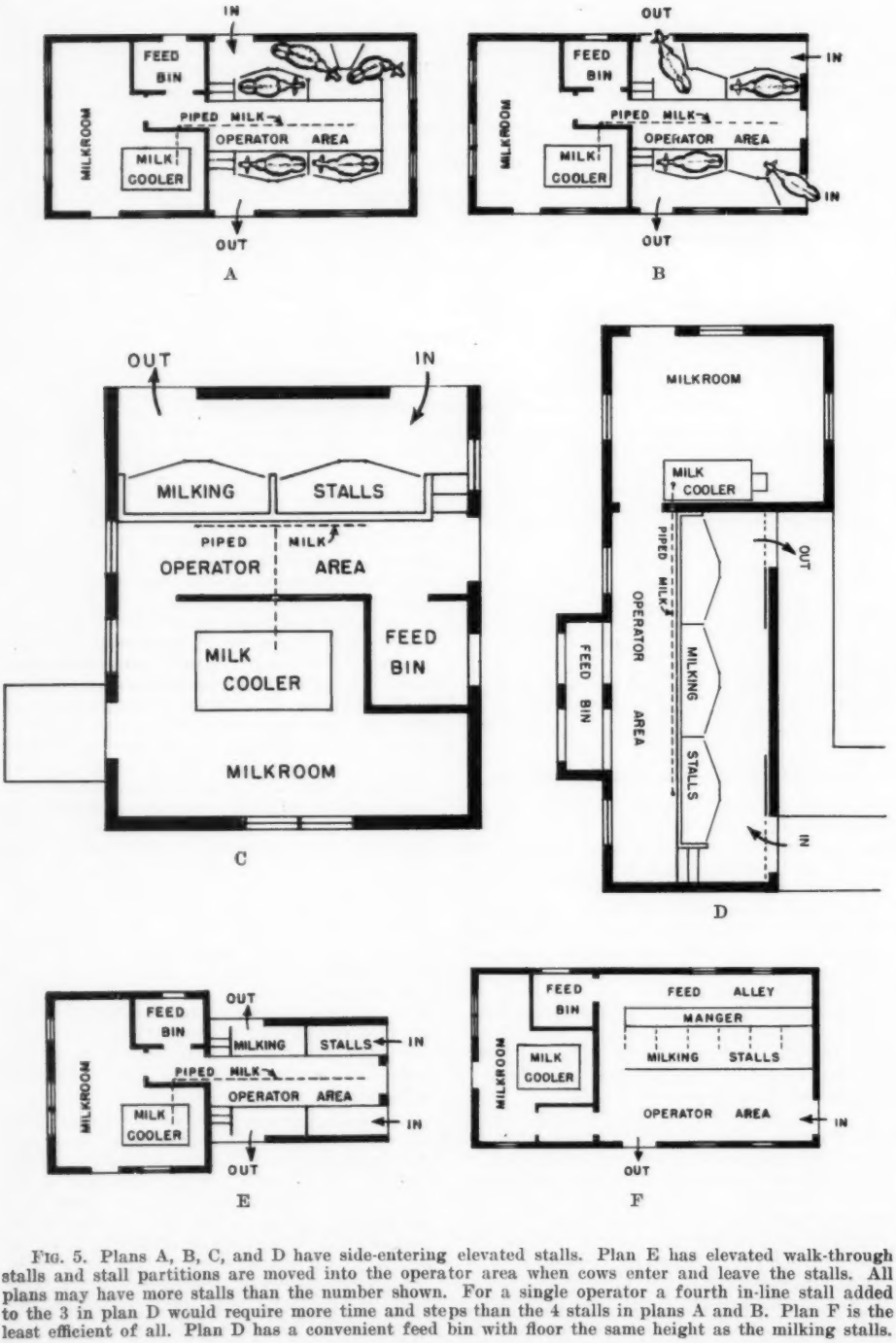


FIG. 5. Plans A, B, C, and D have side-entering elevated stalls. Plan E has elevated walk-through stalls and stall partitions are moved into the operator area when cows enter and leave the stalls. All plans may have more stalls than the number shown. For a single operator a fourth in-line stall added to the 3 in plan D would require more time and steps than the 4 stalls in plans A and B. Plan F is the least efficient of all. Plan D has a convenient feed bin with floor the same height as the milking stalls.

Types of Milking Rooms

There are a number of milking room arrangements which are almost equally satisfactory but are intended for different situations. In general, the elevated stall milking rooms are preferred because they are smaller, cost less, and permit easier and faster milking operations than floor level milking rooms. Figure 5 — A, B, C, D, E — are elevated stall types. As an economy measure, floor level milking stalls may be justifiable where a few stalls in an old barn can be cleaned up, remodeled, sealed off from the rest of the old barn and made to comply with quality milk production regulations. Figure 5F shows a floor level arrangement of this type. In other words it costs less to remodel a few stalls than all the stalls in an old barn to meet the requirements for quality milk production, but an elevated stall farm milking plant is more economical over a period of years if capital is available for building it.

Compare the Costs

A complete loose-housing layout with all necessary equipment will cost from one-half to two-thirds as much as a stall barn completely equipped for the same size herd. From the above table it is evident that about one-half of the farm dairy investment is in dairy service buildings. Studies over a period of years have shown that considerable savings can be made by more careful planning of stall barns and by using more one-story barns, but much greater savings usually can be made in time, labor, and investment with a loose-housing system. By an efficient, well arranged loose-housing plan, the building investment may be lowered by as much as 50%. It is well worth considering.

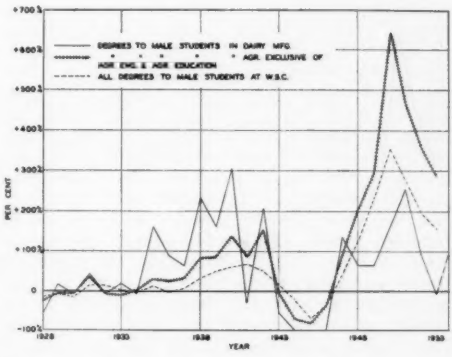
Trends and Challenges in the Training of Dairy Technologists

H. A. Bendixen
State College of Washington

Considerable concern is shown everywhere today about the low enrollment in the field of dairy technology at our colleges. A recent survey by Dr. I. A. Gould of Ohio State University revealed that the total number of degrees in dairy technology granted by colleges in the U. S. dropped 46% from 1950 to 1953. There are, no doubt, numerous reasons for this rather alarming situation. It must be remembered, however, that 1950 was a boom year for the number of all students graduated from American colleges, because of the flood of war veterans taking advantage of government aid for education.

At the State College of Washington a study has been made of the general trend in the number of various graduates at our institution over a longer period of time. The accompanying chart shows the percentage changes in the total number of academic degrees granted to male students during the last 25 years: (1) in dairy manufacturing; (2) in all branches of agriculture, exclusive of agricultural education and agricultural engineering; and (3) in all divisions of the College, using as a base the average number of such degrees granted during the first 5 years of this period, namely, the years of 1928 through 1932. These averages were: 4.2 for dairy manufacturing students, 32.2 for students in agriculture, and 310.2 for all students in the College.

It is interesting to note that during the depression years of the early thirties the number of male graduates in dairy manufacturing increased at a higher rate than the number of male graduates in all of agriculture and in the college as a whole, which is in accord with the common observation that dairy operations usually are most active when times are hard. Dur-



ing World War II, of course, the number of all male graduates dropped to very near zero. During the postwar years the greatest increase in male graduates was in the College of Agriculture as a whole, and the lowest in dairy manufacturing. It is possible that relative prosperity among the farm population, together with the government aid to student war veterans, brought many farm youths to college who otherwise would not have come. Dairy manufacturing, however, seemed to have less appeal than numerous white collar jobs which became avail-

able after the war. The number of degrees granted annually to dairy manufacturing students at W.S.C. averaged 4.3 for the years 1928-1934, inclusive; 10.5 for the years 1935-42; less than 1 during the war period of 1943-46; and 8.9 for the years 1947-53.

Fewer Plants but Greater Need for Technically Trained Men

In spite of the current slump in the butter industry, the demand for dairy products will continue to increase and with it will be greater need for trained dairy personnel. However, the trend has been toward fewer and larger plants. Such a trend is inevitable because of the ability of the larger plants to reduce their per-unit costs by the use of large, continuous-flow processing equipment and because of the opportunity for more effective quality control and merchandising practices. According to U. S. Bureau of Agricultural Economics figures, the number of plants reporting the manufacture of creamery butter and cheese decreased 24% between 1944 and 1950. Wholesale ice cream plants and condenseries decreased 10-15%. Other government figures taken from the Census of Manufacturers showed that from 1929 to 1947 the number of plants manufacturing creamery butter, wholesale ice cream, cheese, and concentrated milk decreased 53.8%, 51.7%, 38%, and 20%, respectively. No figures are available pertaining to market milk processing plants, but many such plants have disappeared in recent years, particularly in the smaller communities, which are now supplied with milk in paper bottles by larger dairies in nearby cities.

With increased mechanization and fewer plants the relative amount of unskilled labor needed decreases. On the other hand, the higher investment in equipment per worker continuously accentuates the need for technically trained workers in order to safeguard the equipment, as well as the products. Data presented in the October, 1953, number of *Food Engineering* show that the number of scientifically trained people employed in dairy plants increased 160% from 1939 to 1950. On the other hand, much more such personnel could be used to advantage in dairy plants, especially in research and development work, for it seems that the amount of money spent in 1951 for research and development amounted to 2% of sales for all manufacturing plants in the country, but only 0.3% for the food manufacturing plants, including the dairy plants. A qualified research staff in any organization may bring returns in the form of quality improvement, savings on equipment, savings in the amount and kind of supplies used, reduction of man hours required, development of new products and new processes, improved methods of merchandising, and in many other ways.

Demands for trained dairy technologists come also from dairy equipment and supply firms, the various inspection and control agencies, government laboratories, and other areas. A great variety of job opportunities exists in the dairy field today, requiring a variety of talents and aptitudes.

College Curricula May Need Revising

It has been said that present dairy technology training is inadequate. The programs definitely need constant revision and supplementation. Of first importance to anyone entering any phase of dairy work, however, is always a sound basic knowledge of the products of the industry. Whether a man is engaged in procurement and buying, production, research, engineering, sales, public relations, or executive work, he first requires a thorough knowledge of the products of his firm to be effective in his work. He needs to know the strong points and the weak points of these products, their food value, their flavor, color and texture characteristics, and their vulnerability to deteriorating influences from the time of production on the farm until consumed. How are the physical, chemical, and bacteriological properties of milk and its products affected by various conditions during transportation, in storage, during processing, in the retail stores, and in the consumer's kitchen? Flavor, appearance, nutritive value, and wholesomeness may be seriously affected by very minute defections. Dairy chemistry, dairy bacteriology, and physics are, therefore, basic in all technical dairy training. Economics, marketing, accounting, psychology of selling and personnel management, refrigeration, mechanics, and public speaking are all important adjuncts to dairy education.

How to Attract More Students into Dairy Technology

Why are the colleges failing at this time to interest more students in dairy technology? Looking at the over-all situation, it will be found that large numbers of our most capable young men are drawn into scientific and engineering fields, and the armed services. Worldwide tensions are resulting in a demand for technical assistance to friendly foreign nations as well as help in solving national and international human and economic problems. As a result, there are not enough trained men available for all of our many technical and professional needs.

To correct the situation more of our high school graduates should be attracted into college work, even though for many of them the currently high union wages are sufficient temptation to cause them to marry early and forego a college education. The choice of a profession presents a formidable problem for any young person today. At best, the high school senior or college freshman can gain a degree of familiarity with only a very small number of ways of

making a living, and he should be given an opportunity for every type of guidance, aptitude tests, and preliminary work experiences to keep him from becoming a square peg in a round hole.

Dairy industry people owe it to themselves and to these young men to inform them of the nature and variety of technical dairy work that exists and to explain to them the importance of this industry in national life, its great size, stability, and numerous ramifications. Many of these young men would be keenly interested and greatly benefited by conducted tours through dairy plants and laboratories. They would benefit from occasional work experiences, if such could be arranged. Above all, however, the need for, and the advantages of, college training from the standpoint of future advancement and a more satisfying life should be emphasized to high school seniors, ambitious employees, and all others who are of college caliber. They should be told that a 4-year course of preparation for a life's work is not too much time, considering the tremendous amount of technical information which has been accumulated during 100 years of development in our industry. Even though wages for untrained help may currently appear very satisfactory, technical training still pays off in the end and will be increasingly important in the operation of our future dairy plants.

A 4-year college course, furthermore, provides an opportunity for the student to determine the area of work best suited to his abilities and needs. He will discover whether his forte lies in plant supervision, in chemical or bacteriological control work, in scientific research, in dairy mechanics, in accounting, salesmanship, personnel work, in the field of inspection, dairy journalism, field work or any other phase of activity in the dairy field. Wherever he finds his greatest interest he will have the best opportunity for success and happiness. Thus, money spent for technical training will usually return great dividends.

Of course, there are always some boys who consider a college education merely a "4-year loaf made with father's dough." For them, a technical course may hold little appeal, but even their time spent in college is likely to be of some benefit. Naturally, these are not the men the industry is especially interested in. Men of the highest caliber are needed, and with a little effort it should not be difficult to interest capable, ambitious young men to prepare themselves for careers in a large, highly respected, and progressive industry.

Industry's Responsibility

If intelligent young men are to be retained in the dairy industry, they must be shown some personal interest when they enter the field. Most graduates realize they still have much to learn about many of the practical phases of plant operation and are willing to serve a period of apprenticeship in the various departments of a plant. Supervisors should shift them from one job to another to further their education and to determine where their talents may be used to greatest advantage. A little interest and guidance from the men above will produce greater effort and increased loyalty to the organization. However, an ambitious young man with advanced scientific training in chemistry or bacteriology can hardly be blamed for becoming discouraged if he is completely forgotten by management while working, perhaps for months on end, in an ice cream sharp room and on the night shift. Such treatment will not improve the present shortage of technical personnel nor will it build loyalty and esprit de corps in any plant. College men should not be engaged merely as cheap help. They constitute the industry's greatest resource for profit and progress in a system of free and competitive enterprise.

The Public Looks at Milk Sanitation

J. C. Flake
Evaporated Milk Association, Chicago

Workers in dairy technology and dairy husbandry have a vital interest in milk sanitation. The quality and safety of milk and dairy products are of prime importance to consumer acceptance and the welfare of the entire dairy industry.

In the sanitary standards work of a major segment of the industry we are constantly evaluating approaches to the problems as well as the activities of all regulatory and dairy industry representatives. Therefore, an extensive survey was conducted among various groups concerned with milk sanitation to determine opinion on the relative importance of various problems, methods of attacking the problems, ways of improving the effectiveness of milk sanitation work, and the qualifications of a milk sanitarian.

Great interest was displayed in the survey, as indicated by the fact that 90% of the questionnaires were filled out and returned. A total of 160 replies were received from representatives of city and state health departments, state departments of agriculture, U. S. Public Health Service, U. S. Department of Agriculture, colleges of agriculture, producer organizations,

fieldmen, editors of farm and dairy periodicals, and other leaders in the dairy industry. It is believed that workers in dairy science will be interested in the replies to some of the questions.

Although the replies are based largely on opinion, they show the thoughts on some key problems by a large number of industry, educational, and sanitation leaders and men who work directly with dairy farmers. In the survey no effort was made to distinguish between the problems of the various branches of the dairy industry.

J. C. Flake

There was agreement among those who replied to the survey that much remains to be done on dairy farm sanitation and that further efforts along this line will not result in an undue increase in the cost of milk or drive many farmers out of milk production. However, over two-thirds of the replies reported that sanitarians have stressed barn and milk house construction details at the expense of producer methods. They concluded that the dairy industry has reached the point that it is now time to reemphasize basic methods of sanitary milk production.

In another question a list of ten items was given with the request that they be rated as to their importance as problems that remain to be solved in dairy farm sanitation. The items considered most important were rated as follows: (1) milking machine cleanliness, (2) cleanliness of other utensils, (3) clean milking methods, (4) milk cooling, (5) mastitis, (6) brucellosis.

The fact that cleanliness of milking machines and other utensils and clean milking methods were rated at the top of the list shows agreement with efforts of industry and regulatory agencies to measure the effect of these items on milk quality by means of the various bacteriological tests and the sediment test.

Over three-fourths of the replies recognized the need of the dairy industry for a reliable rapid platform test for the detection of individual cans of high bacterial count milk so as to permit the rejection of poor quality milk that is not detected by the usual platform tests. It is hoped that this will be accepted as a challenge by dairy scientists to develop a suitable test for this purpose.

Replies to other questions ponted out that:

1. Sanitarians need to do more work at milking time in order to be better acquainted with milk production methods.

2. Farmers in general do not make the same effort to clean utensils and milking machines after the evening milking as after the morning milking.

3. Much of the value of official inspections is lost because of the practice of warning the individual farmer just prior to the visit by the survey officer to his farm. However, opinion was about equally divided as to whether this practice is prevalent.

4. Multiple inspections, each with different requirements, are a serious block to progress. Officials need to coordinate programs, agree upon basic requirements, accept reciprocal inspection, and especially recognize that personal opinions are not standards.

5. Over one-third of the replies indicated that producers do not understand that the basic purpose of the milk sanitarian's work is to improve the quality and maintain safety of the milk supply.

6. Over two-thirds said that dairy farmers do not appreciate the importance of consumer acceptance of milk and dairy products in order to maintain their market.

Another question listed sixteen items for evaluation of dairy farm sanitarians and their work with a request that they be rated as to relative importance. The items considered most important were rated as follows:

1. Knowledge of milk sanitation.
2. Ability to meet people and to get along with them.
3. Selling ideas rather than quoting regulations.
4. Ability to get producers to follow approved practices.
5. Genuine interest in milk quality and safety.
6. Genuine interest in farm people.
7. Willingness to work hard.
8. Ability to help the producer arrive at the right decision rather than merely giving expert advice.

This list of qualifications needed for success shows that the dairy farm sanitarian must be a great deal more than a milk inspector if he is going to accomplish the job.

It is hoped that this material will stimulate interest, ideas, and work on some of the problems in the field of milk sanitation.

VOLUME XXXVII November, 1954 NUMBER 11

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BUTTER

967. The changes in diacetyl and acetoin content and plate count of different butters during storage. F. BOBECK, I. ANDERSON, S. HOFF, and M. JARVIK. Proc. 13th Intern. Dairy Congr., **2**, 517. 1953.

Sour-cream butter was used in expts. in which the salt content (I) and pH were varied. The following types were studied: (1) I 0% pH 4.7; (2) I 0.7%, pH 4.7; (3) I 0.7%, pH 6.0; (4) I 1.3%, pH 4.7; (5) I 1.3%, pH 6.0. The samples were stored at +13° for 42 days and 90 days at –15°. The diacetyl content (II) increase after 14 days in samples 1, 3, and 5 when stored at +13° which may be attributed to the higher activity of the streptococci. No increase was shown in (2) or (4). From 14 to 42 days there was a decrease in (3) and little change in (4). There seems to be little correlation between the content of II and bacteria. The acetoin content (III) increased with storage in (5). In (2) and (4) no change. (1) and (2) showed a decrease. The streptococci were more numerous in (1) and (3) than in (2), (4), and (5). After 90 days storage at –15° II and III were the same in (1), (4), and (5) with a decrease in (2) and (3). H. J. Velander

968. Cold-storage experiments on butter. F. E. VAN HAEFTEN and J. W. PETTE. Proc. 13th Intern. Dairy Congr., **2**, 541. 1953.

Summer and winter butters were used. Organoleptic evaluation, peroxide value, iodine no., fuchsin test for aliphatic carbonyl groups, and the Kreis test were made. There were samples containing no salt, salt, and Cu. High temp. (90°) and low temp. (70-72°) pasteurization were used. The acid values were nearly constant. The peroxide values above 1 correlated with organoleptic tests (I). Fuchsin tests when pos. correlated with I. High temp. pasteurized cream gave the best butter. Salted butter had a lower peroxide value than unsalted. Cu was responsible for a "fishy" flavor. Summer butters keep better than winter butters. To prevent cold storage defects killed yeast cultures, antioxidants, and sterilized milk were added to the cream. No beneficial effect was shown by the addn. of killed yeast, sterilized milk, dodecyl gallate, nondehydroguaiaretic acid, lecithin, and carboxy methylcellulose. Tetraethylthiuram disulfide and tetramethylthiuram disulfide had a beneficial effect. Ultra violet irradiation did not cause a fishy flavor but a linseed oil flavor. Lecithin-free butterfat was used in oxidation expts. at 37°, 14°, and 10°. H. J. Velander

969. Churning in the absence of foam. H. MULDER and J. C. SCHOLS. Proc. 13th Intern. Dairy Congr., **2**, 706. 1953.

A cream-air boundary is generally believed to be necessary for churning. Cream has been churned in the absence of air. The churning time in min. with a Holstein churn, r.p.m. of the agitator, and the percentage of fat in the cream are given. A new type of churn is described. H. J. Velander

970. Oxidation phenomena encountered in the manufacture of butter in cheese dairies. W. RITTER. Proc. 13th Intern. Dairy Congr., **2**, 512. 1953.

Whey cream is high in Cu and as a result butter made from it always develops a fishy odor in storage. The elimination of Cu from cream and butter has not been accomplished. Elimination by biological methods is being studied. Sweet and well pasteurized cream gives the best quality cheese-dairy butter.
H. J. Velander

971. Butter print mold. H. R. COON, SR. and E. J. RAPP (assignors to Lynch Corp.). U. S. Patent 2,691,218. 3 claims. Oct. 12, 1954. Official Gaz. U. S. Pat. Office, **687**, 2: 289. 1954.

Details are given for the construction of a commercial butter printer. R. Whitaker

CHEESE

972. Bitter compounds of cheese. C. W. RAADSVELD. Proc. 13th Intern. Dairy Congr., **2**, 676. 1953.

The residue from aq. exts. of bitter and nonbitter cheese was extd. with $CHCl_3$ which gave concentrates of bitter tasting polypeptides. The hydrolyzates of the polypeptides were studied by paper chromatography. The following amino acids were found; a large quantity of proline, large amts. of glutamic acid, alanine, valine, leucine, and isoleucine; smaller amts. of aspartic acid, threonine, glycine, serine, and phenyl-

alanine. Basic amino acids, tyrosine, tryptophane, and methionine were found in traces. Chromatograms of bitter substances isolated from skimmilk cultures of *Streptococcus liquefaciens* were not quite identical with those from cheese. H. J. Velander

973. The effect of oxidizing salts on the oxidation-reduction potential and ripening of Emmentaler cheese. E. Peltola and M. Antela. Proc. 13th Intern. Dairy Congr., **2**, 729. 1953.

A study was made of the effects of oxidizing salts KNO_3 and $KClO_3$ on the oxidation-reduction potential and ripening of Emmentaler cheese. KNO_3 added alone or with $KClO_3$ raises the EH but $KClO_3$ lowers it. Proteases are not affected by KNO_3, but in large amts. it retards deamination of amino acids by propionic acid bacteria. H. J. Velander

974. Factors influencing the ripening of Edam cheese. C. W. Raadsveld. Proc. 13th Intern. Dairy. Congr., **2**, 671. 1953.

A comparison is made between raw and pasteurized milk cheeses. Studies were made on the initial pH of the curd, the moisture content, salt, H_2O ratio, and storing temp. Pasteurized milk cheese had a higher sol. N and lower amino acid content, and a reduced fat hydrolysis. A low acid curd promotes protein degradation with higher values for amino acids and NH_3–N, than a high acid curd. High moisture cheeses favor greater decomp. of protein when the salt : H_2O ratio is lower than in low moisture cheeses. The amino acid and NH_3–N content increases when the salt conc. is lowered. Fat hydrolysis is not increased by modifying salt and moisture content. Protein and fat decomp. is accelerated by high temp. storage. H. J. Velander

975. Forsoegsmejeriet meddeler: Udbytte og overgangstal ved ostning af maelk fra koer af Roed Dansk Malkerace og Jerseyrace. (The Creamery Expt. Sta. reports: Yield and "recovery numbers" of milk from Red Danish and Jersey cows when made into cheese). Anon. Maelkeritidende, **67**: 727. 1954.

Experiments were conducted to compare yield and "recovery numbers" (the percentage of the milk constituents recovered in cheese) of milk from Red Danish and Jersey cows made into Cheddar and Samsoe cheese and acid precipitated casein.

The "recovery numbers" for protein were about equal for the two kinds of milk and ranged from 74-77. The lowest figure was obtained during the summer when the casein content of the milks was lowest, and the highest figure during the winter when the milks contained the most casein.

No significant difference was found in the "recovery numbers" for fat. The season did not seem to influence the results.

More ash was recovered in cheese made from Jersey milk than from Red Danish. The difference, about 4%, was uniform for the different types of cheese, but more ash was recovered in Samsoe cheese than in Cheddar cheese, probably due to the higher acid developed in Cheddar cheese. Acid precipitated casein contained the least ash of any.

The "recovery numbers" for total solids and s.n.f. showed unexpected results in favor of Jersey milk. More dry matter (2-3%) was recovered from Jersey milk than from milk from Red Danish cows. These results had not been anticipated in view of the individual "recovery numbers."

The correctness of evaluating Jersey milk intended for cheese on the basis of its fat content was doubted. The higher yield and "recovery numbers" were due to the higher fat content of this milk, but a considerable amount of fat always had to be removed to obtain the right proportion of fat to s.n.f. T. Kristoffersen

976. New white cheese good for cooking. Wis. Agr. Expt. Sta., Madison. Ann. Rpt. Bull. 510. 1954.

A new creamy-white, mold ripened cheese known as Nuworld has been developed. This product is very versatile in that it blends well when heated, spreads easily at room temperature, crumbles nicely when cold, and can be used in light colored foods without discoloration. R. W. Hunt

977. New cheese announced. Wis. Agr. Expt. Sta., Madison. Ann. Rpt. Bull. 510. 1954.

D. M. Irvine and W. V. Price have developed a creamy flavored, smooth, soft bodied cheese. This new cheese is reported to resemble process cheese, but is distinct in that it is ripened by bacteria. Ripening takes 1-2 months. R. W. Hunt

978. Gas treatment for cheese possible mold control. Wis. Agr. Expt. Sta., Madison. Ann. Rpt. Bull. 510. 1954.

Ten pounds of methyl bromide per 1,000 cu. ft. of air prevented mold growth after 4 hr. of exposure. More research is being conducted on the effects of bromide residues of treated cheeses. R. W. Hunt

979. Liquid cheese compositions and method of preparing the same. G. C. Schicks and E. F. Klein (assignors to Geed, Inc.). U. S. Patent 2,688,553. 9 claims. Sept. 7, 1954. Official Gaz. U. S. Pat. Office, **686**, 1: 172. 1954.

A liquid or very thin bodied cheese product is made by blending pasteurized cured cheese curd with a hexahydric alcohol. R. Whitaker

980. Curd mill. W. G. SCHULZE (assignor to Damrow Brothers Co.). U. S. Patent 2,690,011. 3 claims. Sept. 28, 1954. Official Gaz. U. S. Pat. Office, **686**, 4: 770. 1954.

A motor driven curd mill mounted on two parallel bars which straddle the sides of a cheese vat. R. Whitaker

981. Marketing cheese curd. ANON. The Milk Prod. J., **45**, 9: 27. 1954.

The Sauquoit Valley Dairy Co., Inc., in Utica, N. Y., has started marketing fresh cheese curd because of a local demand for such a product.

The curd is manufactured from pasteurized whole milk using the same procedure as that used for Cheddar cheese. After milling the curd is chilled with cold water, salted and vacuum packed in 15 lb., 1 lb., and 10 oz. plastic bags. Vacuum packing increases the keeping quality of the curd and prevents mold growth. Refrigeration is necessary to prevent fat separation and development of yeasty flavor.

To develop an expanded market for fresh cheese curd an aggressive sales promotion campaign is necessary. This presents additional problems especially since the cheese curd must be sold at a premium price over the current Cheddar market. J. J. Janzen

CONDENSED AND DRIED MILKS; BY-PRODUCTS

982. Water paint containing enzyme-digested protein. S. M. WEISBERG (assignor to National Dairy Research Laboratories, Inc.). U. S. Patent 2,687,384. 12 claims. Aug. 24, 1954. Official Gaz. U. S. Pat. Office, **685**, 4: 885. 1954.

Casein or soya protein, suitable for use in water type paint, is prepared by hydrolyzing the protein with a proteolytic enzyme in a pH range of 6 to 8.5, to a stage wherein the digested protein is compatible with calcium ions and having good bonding and emulsifying power. R. Whitaker

983. Process of hardening, esterifying, and rehardening artificial protein threads. R. L. WORMELL (assignor to Courtaulds Ltd.). U. S. Patent 2,691,568. 11 claims. Oct. 12, 1954. Official Gaz. U. S. Pat. Office, **687**, 2: 389. 1954.

Artificial thread consisting of a protein, such as casein, is made by extruding a soln. of casein into a hardening bath of formaldehyde and a metal salt. The wet properties are then improved by esterifying the thread in an alcohol bath containing a catalyst and again hardening the thread in a soln. of formaldehyde and a metal salt, such as sodium, magnesium, or aluminum sulphate. R. Whitaker

984. Composition for lithographic plate coatings. V. L. GREGORY (assignor to Gregory & Falk). U. S. Patent 2,690,395. 4 claims. Sept. 28, 1954. Official Gaz. U. S. Pat. Office, **686**, 4: 880. 1954.

A coating for lithographic plates composed of a colloid such as casein, albumin, and a soluble gum; a light sensitive material and a substance which will form an emulsion in water, such as dried milk, soybean powder, etc. R. Whitaker

DAIRY BACTERIOLOGY

985. Studies of some presumptive lactobacilli isolated from the rumens of young calves. S. O. MANN and A. E. OXFORD, the Rowett Research Inst., Bucksburn, Aberdeenshire. J. Gen. Microbiol. **11**, 1: 83. 1954.

Thirteen cultures of gram positive rods were isolated from the rumens of young calves on diets both with and without added aureomycin. Ten of the thirteen isolates proved catalase negative; and of these four were identified as *L. brevis*, three as anaerobic variants of *L. lactis* and three cultures obtained from an aureomycin-fed calf were motile, homofermentative, and mannitol fermenting, but could not be identified with any described species of Lactobacillus. J. J. Jezeski

986. Factors influencing the lactic acid-producing properties of streptococci used in the manufacture of Cheddar cheese. I. Observations relating inhibitory and stimulatory phenomena. G. R. JAGO, Dept. of Agr., Victoria, Australia. J. Dairy Research, **21**, 1: 111. 1954.

Varying degrees of susceptibility to an inhibitory substance present normally in milk were found among single strain streptococci used as cheese starters. The substance is labile to boiling but resists HTST pasteurization, and the addition of as little as 10% of raw milk to autoclaved milk is sufficient to depress acid production. The inhibitory substance is largely associated with the fat globules.

Growth stimulatory substances present in fowl liver, yeast, and separator slime were able to overcome the inhibition in raw milk, and also to increase acid production in autoclaved milk. The stimulatory effect was reduced in direct proportion to the addition of freshly separated cream, suggesting a competitive inhibition between the inhibitory substance and a growth factor common to the stimulatory substances. W. D. Schultze

987. The inhibition of mircro-organisms by raw milk. II. The separation and electrophoretic examination of two different inhibitory fractions. J. E. AUCLAIR and N. J. BERRIDGE, Station centrale de Microbiologie et Recherches Laitieres, I.N.R.A., Paris; and Natl.

Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research, **20**, 3: 370. 1953.

The two substances previously identified in raw milk as lactenin 1 (L_1) and lactenin 2 (L_2) have been separated by low temperature acetone fractionation. Although these fractions were highly active, results of electrophoretic experiments indicate that they should be capable of much further purification.

W. D. Schultze

988. Aroma-producing group 'N' streptococci isolated from Cheddar cheese curd. M. Zielinska and E. R. Hiscox, Natl. Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research, **21**, 2: 238. 1954.

Of 48 strains of group 'N' streptococci isolated from Cheddar cheese curd, all but 2 produced volatile acids and acetoin in varying amounts in milk culture. Four groups, defined on the basis of acetoin production, were characterized culturally and biochemically. Group I closely resembled type strains of *S. diacetilactis* and *S. citrophilus* with respect to strong formation of CO_2, acetic acid, and C-4 compounds in the presence of citrate. They also produced small amounts of acetoin from lactose alone, apparently at the expense of lactic acid. Groups II and III resembled *S. cremoris* HP in low acetoin production, but group II showed similarities in morphological and cultural reactions to *S. lactis*. Group IV produced no acetoin.

W. D. Schultze

989. Methods for the growth and enumeration of anaerobic spore-formers from cheese, with observations on the effect of nisin. A. Hirsch and E. Grinsted, Natl. Inst. for Research in Dairying, Univ. of Reading. J. Dairy Research, **21**, 1: 101. 1954.

New techniques for the isolation and study of anaerobic spore-formers were developed and tested on a collection of various clostridia. Pure strains were isolated by making multiple spot inoculations on agar plates from serial dilutions of the liquid culture. That dilution from which only a few of the drops produced growth was assumed to be sufficiently dilute to give one viable organism per loopful. A clear liquid medium based on yeast-glucose broth was found superior to those in the literature for supporting rapid growth and gas production. A sufficiently low redox potential was provided by the inclusion of 0.05% cysteine, since thioglycollate was found to be toxic for some clostridia. Of various counting methods studied, the dilution method gave the highest counts with the greatest precision, and was easiest to perform.

The effect of nisin on strains of *C. sporogenes, C. butyricum,* and *C. bifermentans* was investigated using the cultural fluid from a nisin-producing streptococcus. Young vegetative cells were more susceptible than older ones, and gas production was inhibited more readily than growth. Spores were more susceptible than vegetative cells, and according to the nature and numbers of the test organism nisin can act either sporicidally or as an inhibitor of germination.

W. D. Schultze

990. The surface microflora of Stilton cheese—normal and slip-coat. M. Brindley, School of Agr., Univ. of Nottingham. J. Dairy Research, **21**, 1: 83. 1954.

The surface microflora of Stilton cheese at the time of scraping was investigated for its relation to the soft, flaky surface defect known as slip-coat. The bacterial and mold populations of normal and slip-coat cheese were not significantly different in magnitude. Total bacterial counts ranged from 3 to 17 billions per gram and were much higher than in samples from the interior of the cheese. The normal and defective cheese had similar ranges of pH at the surface, which were higher than those found in the interior. The more rapid increase in pH at the surface of the cheese may reflect the action of the microflora or possibly the chemical composition of this stratum. At surface pH's below 6.0, yeasts and lactic acid bacteria predominate in the normal cheese; above pH 6.0 diptheroid rods, pigmented cocci, and aerobic sporeformers are more numerous. The mold count at the surface is usually rather low at the time of scraping, being restricted to species of *Penicillium, Mucor,* and *Oospora.* Among pasteurized milk cheese from different dairies wide variations in the surface microflora were found to correlate with the type of coat formed on the cheese.

In contrast to normal cheese, at least 100 millions per gram of *P. vulgaris* were found in 2 of 3 slip-coat samples. In one of these samples *S. liquefaciens* was associated. In the third sample more than a billion per gram of *O. lactis* were present. The possible role of these types in the development of slip-coat was discussed in relation to the physiocochemical environment at the surface of the cheese.

W. D. Schultze

991. Paavisning af forskellige maelkesyrebakteriers fordeling. (Demonstration of the distribution of different types of lactic acid bacteria). C. Lind, Hilleroed, Denmark. Maelkeritidende, **67**: 683. 1954.

A method is given by which an estimate of the distribution and numbers of lactobacilli and streptococci in cheese can be obtained. The method is based on the differences in optimum temperature for growth and acid formation of these bacteria.

Two sets of tubes of sterile milk were inoculated with increasing dilutions of a sodium citrate solution of cheese. One set was incubated at 38° C. for 2 days and the other set at 20-22° C. for 4-5 days. After incubation transfers were made to new sets of tubes and the original tubes titrated. This procedure was re-

peated once or twice. From the titration results the types and numbers of lactic acid bacteria could be estimated.

Emmentaler cheese showed a decrease in *L. helveticus, L. acidophilus,* other lactobacilli and streptococci as it matured, whereas Samsoe cheese showed an increase in lactobacilli but a decrease in streptococci. T. Kristoffersen

DAIRY CHEMISTRY

992. Phosphorus linkages in α-casein. G. E. Perlmann. Nature, **174**, 4423: 273. 1954.

It was shown experimentally that protein contains pyrophosphate bonds and that α-casein contains some of its phosphorus as pyrophosphate and phosphodiesters. Specific enzymes were employed to produce monoesters which were then readily hydrolyzed by the action of phosphomonoesterases. The author also uses her data to calculate the molecular wt. of casein as 31,000 from the pyrophosphate linkages of α-casein. R. Whitaker

993. Colorimetric determination of ascorbic acid. New developments concerning the reaction with diazotized 4-methoxy-2-nitroaniline. M. Schmall, C. W. Pifer, E. G. Wollish, R. Duschinsky, and H. Gainer, Hoffman-La-Roche Inc., Nutley, N. J. Analyt. Chem., **26**, 9: 1521. 1954.

A procedure has been developed which offers good possibilities for the determination of ascorbic acid in media where previously reported techniques could not be successfully applied. When samples of milk, dried milk, and animal feed were tested, either no color or only rapidly fading colors could be produced with the original procedure. When the new technique was applied to these samples relatively stable colors were obtained. B. H. Webb

994. A biological method to detect the presence of some modern preservatives (organic chloro and bromo compounds) in milk. T. E. Galesloot. Proc. 13th Intern. Dairy Congr., **3**, 1200. 1953.

Five hundred ml. of the suspected milk are heated to 50° and 30 ml. of N H_2SO_4 are added. Remove the curd and add 50 ml. of Fe_2O_3 sol. Ext. twice with 250 ml. of Et_2O. Wash the Et_2O ext. several times with 10 ml. H_2O, and evap. to 2 ml. Add the residue to 100 ml. of sterilized skimmilk. Add 2 ml. of Et_2O to another flask as a control. Incubate 2-3 hr. at 37°, and inoculate with 0.005 ml. of skimmilk culture of *S. lactis*. The acid is measured by titration. Bromoacetic acid and bromacetic ester is easily detected at 0.001%. Trichloronitromethane, Conservatol, Microsol, and Steriline are detected at 0.005-0.02%. The addn. of these compds. is indicated by a delayed acid production. H. J. Velander

995. Rapid method for determination of solids-not-fat in milk. A. G. Leggatt. Can. Dairy and Ice Cream J., **33**, 4: 36. 1954.

In a refinement of previous work the author has developed a rapid method for determination of solids-not-fat in milk. Briefly the method consists of warming a sample of milk to 60 deg. F., and diluting this milk (1:9) in a 100-ml. volumetric flask. Five ml. of this milk is transferred to a white casserole and diluted with 20 ml. water at 60 deg. F. Two drops of O-phenanthroline ferrous complex indicator are added and the mixture titrated with ceric sulphate at a rate of 1 ml. per 10 sec. until color is discharged. After one min. (the color having returned) the sample again is titrated until the solution remains gray. The ml. of ceric sulphate can be converted to percentage of solids-not-fat by means of a table or by means of an equation: $Y = 1.37 + 4.18X$, where Y = S.N.F. and X = ml. ceric sulphate. On 36 samples this method gave a difference of 0.1% or less on 75% of these samples. The deviations between this method and standard method showed significant agreement. H. Pyenson

996. The measurement of solids in milk by oxidimetry. A. G. Leggatt. Can. Dairy and Ice Cream J., **33**, 3: 30. 1954.

A titration method of determining the total solids in milk has been developed using .01 N ceric sulphate in N sulphuric acid. The data obtained yielded a 91% agreement between the observed and calculated values for total solids. Later studies indicate temperature and time influence the oxidation-reduction reactions and present a problem for future study. The method appears to hold possibilities and further work will be required in the development of technique. H. Pyenson

997. Paper chromatographic determination of the amino acids in cheese. B. Lindquist, T. Storgards,. and B. Goransson. Proc. 13th Intern. Dairy Congr., **3**, 1254. 1953.

When buffered solns. and papers were used the separating power of the one-dimensional technique is as great or greater than two-dimensional. The procedure is given. In eight types of cheese studied, threonine, alanine, valine, methionine, leucine, lysine, and glutamic acid were always present. Aspartic acid, serine, glycine, and isoleucine were sometimes absent. Tyrosine, proline, tryptophan, arginine, histidine, tyramine, glutamine, asparagine, and phenylalanine are usually present. Hydroxyproline and cystine are rarely or never found. H. J. Velander

998. Determination of the volatile acids in cheese by paper chromatography. B. Lindquist, T. Storgards, and B. Goransson. Proc. 13th Intern. Dairy Congr., **3**, 1250. 1953.

One-dimensional technique is used because of the greater possibility of identifying spots. The

procedure is described. AcOH, formic, and lactic acids were found in ten types of Swedish cheese. Butyric acid is normally present and valeric acid may also be found in Blue Cheese. Port Salut and Camembert Cheese are generally low in acid because of utilization by the flora.

H. J. Velander

999. The determination of bromates in milk, whey, and curd with regard to their use in cheesemaking. G. Schwarz and H. Mumm. Proc. 13th Intern. Dairy Congr., **3**, 1331. 1953.

An iodometric method is used for detg. the amt. of ($KBrO_3$-KNO_3) added to milk for cheesemaking. A study of Tilset cheese showed that 93% ($KBrO_3$-KNO_3) stayed in the whey, while 6% remained in the curd.

H. J. Velander

1000. Developing tests for foreign fats. Anon. The Milk Prod. J., **45**, 9: 34. 1954.

The use of substitute fats, singly or in combination with butterfat, has taken on major proportions. This has emphasized the need for additional research to develop an adequate test to detect adulteration and thus protect consumers against fraud and deception.

A survey of the literature of the properties of fats and oils indicates that no single property can be used as the sole criterion for identifying all possible mixtures of edible fats. Research is being conducted to develop a method which can be used to detect all of the types of fats that are being used in imitation of butterfat.

J. J. Janzen

DAIRY ENGINEERING

1001. Valve-actuating mechanism. A. F. Buzas. U. S. Patent 2,684,826. 3 claims. July 27, 1954. Official Gaz. U. S. Pat. Office, **684**, 4: 857. 1954.

Milk cans are placed on a platform which is balanced in a raised position by means of counter weights equal to the weight of the can and desired net contents. A lever is pulled to open a valve in the product supply line and the container is filled. When the weight of container approaches the desired weight, the platform is depressed, and an actuating device closes the supply valve.

R. Whitaker

1002. Machine for inserting containers or bottles in cases, crates, or cartons. S. J. Lewis. U. S. Patent 2,684,800. 17 claims. July 27, 1954. Official Gaz. U. S. Pat. Office, **684**, 4: 849. 1954.

Details are given for a machine which will pick up four paper or glass milk bottles at a time from a conveyor line and place them in cases which proceed at a synchronized rate on another conveyor.

R. Whitaker

1003. New processes and equipment, etc. R. P. Dunmire. U. S. Patent 2,685,522. 16 claims. Aug. 3, 1954. Official Gaz. U. S. Pat. Office, **685**, 1: 176. 1954.

Milk at 100° F. is deaerated and sterilized by spraying it from a pressure of 5,000 p.s.i. into a vacuum of about 29.75 in. Hg. This reduces the temp. to 40°. To facilitate processing in this manner, an apparatus is described, consisting of a rotor, for creating the spray, rapidly rotating in a cylindrical vessel under high vacuum, so shaped and designed to collect the sprayed liquid particles in a film and then deliver the cooled liquid to atmospheric pressure.

R. Whitaker

1004. Double-headed commercial ice cream freezer. H. F. Swenson. U. S. Patent 2,687,019. 21 claims. Aug. 24, 1954. Official Gaz. U. S. Pat. Office, **685**, 4: 783. 1954.

Two ice cream freezers, powered by separate motors, have refrigeration supplied by a single compressor. A system of electrical circuits is provided which permits operation of each freezer, singly or in combination.

R. Whitaker

1005. Container structure. R. H. Innes (assignor to Perga Containers Ltd.). U. S. Patent 2,687,840. 9 claims. Aug. 31, 1954. Official Gaz. U. S. Pat. Office, **685**, 5: 1084. 1954.

A method of forming the top of a Perga type paper milk bottle which produces a pouring spout when the consumer squeezes the sides of the top in a certain manner. The pouring lip remains protected until the spout is formed.

R. Whitaker

1006. Feed roller attachment for butter print machinery and the like. H. H. Doering. U. S. Patent 2,687,830. 5 claims. Aug. 31, 1954. Official Gaz. U. S. Pat. Office, **685**, 5: 1081. 1954.

A spiral type conveyor is described for propelling butter from a hopper to a print-forming device.

R. Whitaker

1007. Apparatus for treatment of cream and milk. C. A. S. Thorstensson-Rydberg. (assignor to J. R. Andersson & Co. Aktiebolag). U. S. Patent 2,688,468. 3 claims. Sept. 7, 1954. Official Gaz. U. S. Pat. Office, **686**, 1: 149. 1954.

A churn for making butter consisting of a cylindrical vessel with a built-in baffle, which rotates and may be tilted for discharging the gathered butter. A portion of the cylinder is jacketed to permit adjusting the temp. of the dairy product by a heating or cooling medium.

R. Whitaker

1008. Freezer converter. C. W. Merrill. U. S. Patent 2,689,113. 4 claims. Sept. 14, 1954. Official Gaz. U. S. Pat. Office, **686**, 2: 387. 1954.

Details are given for the construction of a freezer consisting of a drum rotating in a cylinder of slightly larger diameter. The mix entering at the inlet is immediately thrown against the refrigerated walls of the cylinder by centrifugal force. Blades for scraping the cylinder wall are mounted in recesses in the drum, while in another recess the mix is whipped out of contact with the refrigerated cylinder wall and then discharged from the freezer. R. Whitaker

1009. Method and arrangement for homogenization of liquids. S. H. B. ZACHARIASSEN (assignor to Aktiebolaget Separator). U. S. Patent 2,690,900. 14 claims. Oct. 5, 1954. Official Gaz. U. S. Pat. Office, **687**, 1: 149. 1954.

Milk and other dispersions are given an homogenization treatment by pasage through a rapidly revolving bowl equipped with baffles in the form of cone-shaped dises.

R. Whitaker

1010. Engineering the plant of the future. J. H. FORSLEW, Carnation Co., Los Angeles, Calif. Ice Cream Rev., **38**, 2: 48. 1954.

A site of adequate size is one of the essential requirements of the modern dairy plant. The site must not only be large enough to accommodate the plant proper, but it must provide for adequate parking area and such other installations essential to the conduct of the business. The plant should be located accessible to traffic on three sides with the office and visitors' parking and landscaping on the fourth side.

The modern plant should be made attractive to the public and be equipped with facilities which will encourage people to visit the plant and view its operation. It should be large enough to provide adequate space with provision for expansion in case additional space is required.

The suggested design provides for a basement warehouse which serves as a practical location for service lines. The processing areas are located on the first floor, and the second floor will house the offices, drivers' room, hostess room, lunch rooms, and toilet and locker rooms. The entire plant will be mechanically heated, ventilated, and in many cases air conditioned. A centrally located room housing equipment for cleaned-in-place equipment is essential to the modern plant.

In future operations it is contemplated that much physical handling of the product and containers will be reduced or eliminated. This will be accomplished by loading products directly into mechanically refrigerated trucks. The trucks will be refrigerated with self-contained freon packaged units built into the truck bodies and equipped with 440-volt, three-phase motors.

Some changes in equipment which will affect plant design include: (1) farm bulk cooling of milk, (2) use of rectangular storage tanks which will be cleaned in place, (3) faster paper bottle fillers, and (4) use of automatic casers and case stackers.

The ice cream processing room will house all the processing equipment. Packaging supplies will come either from the first floor warehouse or from the basement. Freezers of larger capacity and larger storage tanks will be used in the future. Through the use of improved hardening tunnels it is anticipated that the product will go directly from the freezers to the trucks.

Improvements are needed in equipment for producing novelties to reduce labor requirements and also the floor area occupied by the equipment. W. J. Caulfield

DAIRY PLANT MANAGEMENT AND ECONOMICS

1011. Dairy production and price trends in New Jersey. J. W. CARNCROSS and A. G. WALLER, N. J. Agr. Expt. Sta., New Brunswick. 1954.

Subjects covered in this publication include Heifer numbers and milk production per cow in New Jersey. Prices paid for milk, feed, hay, wages, milk cows and beef cattle are also discussed. These items are listed in table or graphic form. R. W. Hunt

1012. Can washing and sterilizing process. F. M. SCALES. U. S. Patent 2,677,630. 5 claims. May 4, 1954. Official Gaz. U. S. Pat. Office, **682**, 1: 210. 1954.

A process for washng milk cans consisting of the following steps: (1) exterior can rinse of water and detergent, (2) interior can wash of a noncorroding alkaline synthetic organic detergent solution containing a water soluble fat solvent, (3) draining the cans, (4) sterilizing the interior with a spray of a solution of a cationic quaternary ammonium halogen compound and a rust inhibiting compound selected from the group consisting of alkali-reacting amine and ammonia. None of the solutions exceed 150° F. in temp. R. Whitaker

1013. Can washing and liquid feeding device. C. M. MOORE (assignor to Cowles Chemical Co.). U. S. Patent 2,687,136. 4 claims. Aug. 24, 1954. Official Gaz. U. S. Pat. Office, **685**, 4: 817. 1954.

Construction details are given for a straight line milk can and lid washer, having a measured amount of detergent solution sprayed by a steam jet into the inverted cans as they are moved along over a series of nozzles.

R. Whitaker

1014. Dairy plant labor. ANON. The Milk Prod. J., **45**, 9: 29. 1954.

A dairy plant labor survey was conducted by the Colorado A & M College in 45 plants rang-

ing in size from 3,200 employees to 9 employees and representing cross-country sampling. Half the plants had labor unions and the large plants were unionized except in the South. Wage scales ranged from $30 to $60 in the South and $57 to $115 per week in the coastal states. Wages ranged higher in nonunion dairies than in union dairies with a few minor exceptions. About half of the plants had disability benefits and 30% had retirement benefits largely made up of those plants having unions. Two plants had profit-sharing plans. About 90% of the plants paid their driver-salesmen on a commission or commission plus guarantee basis. Most plants paid overtime for holiday work. College graduates employed were 254 out of a total 8,214 employees, a ratio of 1 to 32. One plant employed 154 people with no college graduates; another employed 52 persons, 7 being college graduates. The maximum number of hours worked by employees was 54 hours per week and the minimum 40 hours.

The chief employee problems voiced by those who returned questionnaires were: (1) Education and training of new employees (30 plants). (2) Lack of employee interest in work (25 plants). (3) Incompetence and lack of willingness to accept responsibility (22 plants). (4) Obtaining trained personnel for the job to be done (19 plants). (5) Driver-salesmen incompetence (8 plants). J. J. Janzen

1015. Who buys dairy products—and why? ANON. The Milk Prod. J., **45**, 9: 32. 1954.

ADA's second survey on "Who buys dairy products and why?" made in the spring of 1954 showed the following:

(1) NFDMS is almost equally popular with all economic groups, but usage of the product varies greatly. Cooking is the most popular usage whereas a larger volume is consumed as beverage. Sharp differences exist in regional usage: South 30.7%, West 24.9%, Northeast 24.3%, North Central 18.3%.

(2) Ten times as many people use evaporated milk for cooking as use it in baby's formula, however, a much greater volume of this product is consumed in preparing the latter. Evaporated milk is most popular with lower income groups. The South and West regions of the country show the greatest use of evaporated milk.

(3) About one family in five surveyed uses skimmilk, mostly for drinking and it is most popular with the upper economic group.

(4) Consumers use two-thirds of their butter as a spread, while less than half of the oleo is used for that purpose. The survey indicates that butter should be promoted as a prestige product in cooking and baking. The survey showed that butter packaging needed improvement to be competitive with oleo. Since four out of five housewives use recipes and half of them get them from food packages, the survey suggests that recipes should be printed on butter packages to promote prestige cooking.

(5) Nine out of ten families eat cheese and seven out of ten families have cheese in their home. Results indicated that "point-of-sale merchandising" and "store displays" would be helpful to stimulate impulse buying.

J. J. Janzen

1016. Outer-market distribution of milk in paper containers in the North Central Region. Purdue Univ. Agr. Expt. Sta., Lafayette, Ind. Sta. Bull. 600. 1953.

This study was based on data for May, 1952, from 588 milk plants in the North Central Region that were equipped to package milk in paper containers. The volume of milk sold in paper containers outside local markets amounted to 12% of all bottled and packaged milk distributed in the region. Eighty-three of the operators who used paper containers had outer-market sales. Distance of shipments ranged from 8-450 miles, with 23% of distribution reporting distances over 100 miles. In general, as the distance of shipment increased, direct distribution by the plant declined. Improved transportation facilities and refrigeration make it feasible to haul packaged milk over long distances. Moreover, milk in paper containers is better suited to long hauls than milk in glass because it is lighter, more compact, and stays cool longer. R. W. Hunt

1017. Tank-truck assembly of milk for New Hampshire. J. R. BOWRING, N. H. Agr. Expt. Sta., Durham. Station Bull. 410. 1954.

The problem of converting to bulk-tank pickup of milk in New Hampshire is discussed. In order for a dealer to convert to the bulk system a large percentage of the producers must purchase bulk tanks. This forces the small producer to shift to another dealer, purchase a tank, or discontinue business. Since few small producers can afford the bulk tanks, it will mean competition by the dealers for the large bulk-tank producers. This situation could result in many serious adjustments for all concerned. R. W. Hunt

1018. Fluid milk utilization and disposition. Rhode Island, 1950-51. J. R. TEDFORD and F. R. TAYLOR, R. I. Agr. Expt. Sta., Kingston. Bull. 318. 1954.

During the period of this study, the number of firms varied from 241-253. Of these firms, 82% handled less than 1,000 qt. daily, representing 27% of the total milk receipts. About 4% handled more than 5,000 qt. daily. Class I milk accounted for 94% of the milk sold and 77% of this quantity was sold as Grade B, of which 70% was pasteurized and 30% homogenized. Grade A milk represented 20% of the sales, with 69% being pasteurized and 31% homogenized. Flavored drink accounted for less than 3% of total sales. Sixty-two per cent of

the fluid milk was sold through retail routes and 38% through wholesale routes. Between 1950 and '51, total market receipts were increased by more than 4 million quarts. The upward shift in milk receipts and utilization activities was accompanied by a rise in the minimum level of prices, both those paid to the producer and by the consumer. R. W. Hunt

1019. How to handle employee grievances. J. Bedford. Ice Cream Rev., **38**, 2: 108. 1954.

A three step formula for handling employee grievances is suggested. 1. Sympathize with the employee so that he will feel he has found a friend in whom he can confide his troubles. 2. Listen while the employee discusses his grievance in order that the real cause of the trouble may be determined and a plan for the solution of the problem developed. 3. Smile—this makes the employee feel he has found a sympathetic friend and he will be in a receptive mood to go along with the suggested solution to the problem.

The whole discussion concerning the grievance must be conducted on a sincere, friendly basis if the desired results are attained.

W. J. Caulfield

FEEDS AND FEEDING

1020. Too much limestone lowers digestibility of silage. N. F. Colovos, H. A. Keener, A. E. Teeri, and H. A. Davis, N. H. Agr. Expt. Sta., Durham. Ann. Rpt. Station Bull. 409. 1954.

Experiments conducted in 1952 and again in 1953 showed that the feeding of 100 grams of ground limestone daily reduced protein digestibility 6-8%. Fifty grams showed no detrimental effect. R. W. Hunt

1021. Influence of silage quality on cheese milk and cheese quality. K. E. Thome and P. Swartling. Proc. 13th Intern. Dairy Congr., **2**, 69. 1953.

The higher the spore content of the silage the greater the chance of blown cheese due to the fermentation of butyric acid. This is influenced by production technique, use of $NaNO_3$, changes in acid production, moisture and pH.

H. J. Velander

1022. The malty smell and taste of milk and butter. E. Hobinger. Proc. 13th Intern. Dairy Congr., **2**, 41. 1953.

Milk from cows which had been fed excessive amts. of rejects from the cider and fruit-packing industry developed the flavor after natural acidification took place. H. J. Velander

HERD MANAGEMENT

1023. Teat cup inflation. E. Shurts. U. S. Patent 2,687,112. 5 claims. Aug. 24, 1954. Official Gaz. U. S. Pat. Office, **685**, 4: 810. 1954.

A teat cup for a vacuum-operated milking machine is described, having a rigid outer wall and a flexible inner tube, formed to make two cells, one above the other, when the teat is inserted. R. Whitaker

1024. Device for use in testing milking machine teat cup inflations. R. S. Passmore. U. S. Patent 2,687,040. 3 claims. Aug. 24, 1954. Official Gaz. U. S. Pat. Office, **685**, 4: 789. 1954.

A spring controlled pivoted pointer which indicates the effectiveness of the pulsations of a vacuum-operated teat cup. R. Whitaker

1025. Milking machine timer. J. A. Brewer. U. S. Patent 2,687,773. 4 claims. Aug. 31, 1954. Official Gaz. U. S. Pat. Office, **685**, 5: 1064. 1954.

Construction details are given for a clock-operated device for discontinuing the pulsations of a vacuum-operated milking machine at the completion of the milking process.

R. Whitaker

1026. Milking parlor stall. R. G. Ferris (assignor to Starline, Inc.). U. S. Patent 2,688,309. 5 claims. Sept. 7, 1954. Official Gaz. U. S. Pat. Office, **686**, 1: 103. 1954.

A design for a cow stall. R. Whitaker

1027. Means for and method of destroying grubs on cattle. E. C. Pierce. U. S. Patent 2,688,311. 1 claim. Sept. 7, 1954. Official Gaz. U. S. Pat. Office, **686**, 1: 104. 1954.

A method is described for eliminating the grubs which frequently inhabit the hides of cattle, consisting of starving the grubs after they emerge from cysts by blanketing the animal over the infected area and imprisoning them without food during their life span.

R. Whitaker

1028. Pressure release valve for milk hose of milking machines. H. A. Heckendorf (assignor to International Harvester Co.). U. S. Patent 2,690,734. 8 claims. Oct. 5, 1954. Official Gaz. U. S. Pat. Office, **687**, 1: 101. 1954.

A pressure release valve, installed in the milk line of a vacuum operated milker adjacent to the receiving vessel, is maintained in a closed position when a negative pressure is maintained in the milk line and in an open position when a positive pressure exists.

R. Whitaker

1029. Milk tank cooler. R. Markley (assignor to Dairy Equipment Co.). U. S. Patent 2,690,061. 4 claims. Sept. 28, 1954. Official Gaz. U. S. Pat. Office, **686**, 4: 784. 1954.

An insulated cabinet for cooling and storing milk on farms, consisting of a tank suspended within the cabinet, sprays for distributing a film of chilled water down the outside walls of

the tank, an opening in the top of the cabinet for introducing the milk, and connected with a deflector for directing the milk down the inside wall of the tank for rapid cooling.
R. Whitaker

1030. Milking structure. H. Petri. U. S. Patent 2,689,546. 13 claims. Sept. 21, 1954. Official Gaz. U. S. Pat. Office, **686**, 3: 575. 1954.

A milking stall arrangement consisting of two parallel stalls with space for an attendant between. A third stall across the back, equally accessible to the operator, is used for washing and preparing the cows for milking in one of the two milking compartments. R. Whitaker

1031. Calf puller. R. M. Minock. U. S. Patent 2,689,569. 7 claims. Sept. 21, 1954. Official Gaz. U. S. Pat. Office, **686**, 3: 582. 1954.

A device for facilitating calving.
R. Whitaker

1032. Dairy farm bulk milk collection. W. H. Haskell, Klenzade Products, Beloit, Wis. Guernsey Breeders' J., **92**, 3: 308. 1954.

The following are listed as necessities in the installation of bulk milk tanks on farms: (1) A tank of larger volume than immediately necessary should be installed. (2) The tank should be located to be accessible for cleaning and loading. (3) The tank should be level and gauged by rod or transparent tube to accurately measure the contents.

The cleaning and sanitizing of farm bulk tanks is summarized as: (1) Flush with lukewarm water immediately after tank is emptied. (2) Use ounce of alkaline detergent in 3 gallons of water at 125° F. Hand brush the entire surface. Every three or four days use a balanced organic acid detergent at the same rate instead of the alkaline detergent. (3) Drain cleaning solution and rinse with hot water, not over 145° F. (4) Drain dry with lid closed. (5) Sanitize with liquid sodium hypochlorite solution (200 p.p. million) just before use.

The tank truck should be similarly cleaned after and before use. A. R. Porter

1033. A comparison of the bulk and can systems for handling milk on farms. P. E. Johnson, H. C. Olson, and R. L. Von Gunten, Okla. Agr. Expt. Sta., Stillwater. Bull. No. B-436. 1954.

This report shows the raw milk used in this study had an average bacterial count when handled in bulk, to be 17,400 as compared to 54,700 for milk handled in cans. After pasteurization, the bulk milk had a count of 107 as compared to 143 for the milk in cans. Less milk was lost as a result of bulk handling because the weight of the milk is determined on the farm. An average loss of 0.32 lb. of milk per can was determined. This study indicated it takes about ¼ the time to handle bulk milk as an equivalent amount in cans.
R. W. Hunt

1034. Dairy cattle breeds. J. B. Parker, U.S.D.A., Washington, D. C. Farmers' Bull. 1443. 1954.

How to choose a breed of dairy cattle, along with the outstanding characteristics of the five major breeds, is presented. R. W. Hunt

1035. A portable milking barn. G. L. Nelson, G. W. A. Mahoney, and J. I. Fryrear, Okla. Agr. Expt. Sta., Stillwater. Bull. B-420. 1954.

Because of an increasing percentage of tenant dairy farmers in Okla., a portable milking barn was developed. This type building enables the migrant or beginning farmer to enter into the dairy business without the large capital investment of a permanent barn. Grade A permits are being issued for milk produced under these conditions. Detailed plans and specifications are included. R. W. Hunt

ICE CREAM

1036. Ice cream making plate. L. M. Kurtz (assignor to General Electric Co.). U. S. Patent 2,686,404. 6 claims. Aug. 17, 1954. Official Gaz. U. S. Pat. Office, **685**, 3: 552. 1954.

A device is described for installing in a home refrigerator for making ice cream. The mix is spread on a flat refrigerated surface and scraped off after freezing by hand by a broad spatula-like scraper to produce a homemade ice cream of smooth texture. R. Whitaker

1037. Ice cream cone vending machine. H. Heinig. U. S. Patent 2,687,824. 7 claims. Aug. 31, 1954. Official Gaz. U. S. Pat. Office, **685**, 5: 1080. 1954.

Details are given for the construction of a vending machine for ice cream cones. The cones are suspended on pins which protrude from a vertical conveyor and are discharged one at a time into a chute as the pins engage a cone-releasing mechanism at the bottom of the enclosing housing. R. Whitaker

1038. Floating ice cream parlor. J. W. Bedwell. U. S. Patent 2,689,986. 3 claims. Sept. 28, 1954. Official Gaz. U. S. Pat. Office, **686**, 4: 763. 1954.

Tables and chairs for customers consuming ice cream, etc., are mounted on a merry-go-round type of structure. R. Whitaker

1039. Method of making variegated ice cream and the like. N. I. Peyton (assignor to Joe Lowe Corp.). U. S. Patent 2,689,537. 4 claims. Sept. 21, 1954. Official Gaz. U. S. Pat. Office, **686**, 3: 572. 1954.

Ice cream and a flavoring material from a continuous freezer are passed through this

device under pressure, which gives a spiral motion to the crudely blended materials. Release of the pressure provides uniform distribution of the flavor through the mass of ice cream. R. Whitaker

1040. Apparatus for coating ice cream in cones. W. J. Habgood (assignor to Eskimo Pie Corp.). U. S. Patent 2,689,544. 2 claims. Sept. 21, 1954. Official Gaz. U. S. Pat. Office, **686**, 3: 574. 1954.

The ice cream in factory filled cones is coated with nuts, confections and other granulated materials in this machine. The inverted cones are suspended over a blanket holding the coating material. Paddles beneath the blanket pat the blanket and toss the coating upward around the cones. R. Whitaker

1041. Method of making and packing ice cream sundaes. V. V. Bond (assignor to Portion Pack, Inc.). U. S. Patent 2,689,798. 1 claim. Sept. 21, 1954. Official Gaz. U. S. Pat. Office, **686**, 3: 648. 1954.

A depression is made in ice cream contained in individual cups and then filled with a fruit or other flavoring material. Over each cup is laid a sheet of paper, plastic, film, etc., perforated in such a manner that a disc slightly larger than the top of the cup will remain when the sheet is torn along the perforations after the cup and contents are hardened. The cups are packed tightly in tubular shaped cartons, which bend the overhanging cover material down around the side of the cup.

R. Whitaker

1042. Analysis of the 1953 volume in mellorine. Anon. Ice Cream Trade J., **50**, 8: 12. 1954.

Mellorine type frozen dessert sales in the United States in 1953 totaled 24,494,000 gal., representing about 100% increase over 1952 sales. Production of 10,870,000 gal. in Texas accounted for almost half of the total volume in 1953. The mellorine gallonage in the 9 states where it is made represents about 11% of all products manufactured, ranging from about 1% in Alabama to about 33% in Texas.

W. H. Martin

1043. The industry sold 750,000,000 gallons of ice cream, sherbets, mellorine, and related products last year, according to the International's sales index. Anon. Ice Cream Trade J., **50**, 9: 12. 1954.

Total sales of ice cream and related products in the United States increased 4.22% in 1953, bringing the total output to 747,763,000 gal. Sherbet sales were up 22.2%, ice milk increased 24.8% and water ices 3.7%.

W. H. Martin

MILK AND CREAM

1044. Reactivation of milk phosphatase following heat treatment. II. R. C. Wright and J. Tramer, United Dairies Research Labs., London. J. Dairy Research, **20**, 3: 258. 1953.

The degree of reactivation of milk phosphatase showed considerable variation that was due to the different milks studied. No correlation was found between the level of reactivation and the fat content of the milk, the content of added ascorbic acid, small additions of metallic ions or amino acids, or original phosphatase content of the milk. Aging the raw milk at high atmospheric temperature increased the subsequent level of reactivation; evidence points to this being a result of bacterial growth. Removal of oxygen or introduction of reducing systems of milk or bacterial origin usually yielded marked increases in reactivation of good quality milks.

Reheating shocked milks over a wide range of conditions in all cases diminished reactivation. This effect was proportional to time but not to the temperature of reheating, as the effect showed minima at 70 and 95° C. These curves suggest the interaction of a destructive and a creative factor for reactivation.

No reactivation occurred in milks in which the soluble milk proteins had been completely denatured, as measured by the Aschaffenburg turbidity test. Possible explanations of the reactivation of milk, based on present concepts of enzyme structure were considered.

W. D. Schultze

1045. The correlation between butterfat and protein. O. Stuber. Proc. 13th Intern. Dairy Congr., **2**, 301. 1953.

A certain relation exists between butterfat and protein content of the milk, but it fluctuates widely. The determination of the butterfat is not a good indication of the protein content and can not be used in place of the determination of protein. H. J. Velander

1046. Learn why some milk resists oxidized flavor. Wis. Agr. Expt. Sta., Madison. Ann. Rpt. Bull. 510. 1954.

H. H. Sommer and Rene Riel report that milk resistant to "oxidized" flavor has a high content of proteose-peptone nitrogen. Milk can be treated with a small amount of a proteolytic, or protein-splitting, enzyme to make it resist oxidized flavor. It was also learned that typical oxidized flavor is due to phospholipids breaking down into unsaturated ketones.

R. W. Hunt

1047. Dispenser for single service milk cans. W. Tamminga (assignor to Monitor Process Corp.). U. S. Patent 2,686,613. 6 claims. Aug. 17, 1954. Official Gaz. U. S. Pat. Office, **685**, 3: 613. 1954.

Milk in individual cans is stacked in a hori-

zontal rectangular shaped refrigerated container. The cans are dispensed one at a time through an outlet situated opposite the bottom row of cans. R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

1048. Nutritive value of milk. Factors affecting the nutritive value of cow's milk. J. N. Bixby, A. J. Bosch, C. A. Elvehjem, and A. M. Swanson, Depts. of Biochem. and Dairy and Food Ind., Univ. of Wis., Madison. J. Agr. Food Chem., 2: 978. 1954.

Experiments were conducted with albino rats to study the effect of season, pasteurization, further heat treatment, and other modifications upon the nutritive value of cow's milk. Weanling rats maintained on a cow's milk diet for 6 weeks grew well, and females maintained for a longer period of time reproduced and lactated normally through three generations. No seasonal variation was observed in the nutritive value of raw, pasteurized, or homogenized milk, and neither pasteurization nor homogenization had an adverse effect upon its nutritive value when growth, reproduction, and lactation performance were used as the criteria of measurement. Young rats, however, maintained on a mineralized cow milk diet had a tendency to accumulate excess fat in their livers. This fatty liver condition was found to be directly related to the butterfat content of the milk diet, but was not related to season, pasteurization, or a deficiency of lipotropic factors. S. Patton

1049. Stability of vitamin A and carotene in butter. M. Antila. Proc. 13th Intern. Dairy Congr., 3, 1439. 1953.

Very little loss of these compds. occur during storage of butter prepared in a number of ways. The pH varied from 4.8 to 6.8.

H. J. Velander

1050. The biological and physiological properties of continuously sterilized milk. W. Fuchs. Proc. 13th Intern. Dairy Congr., 3, 1018. 1953.

Vitamins and nutritive elements are preserved to a large degree by heat sterilization to 150° as shown by biological expts. All the phosphatase present is not inactive. H_2O_2, $CHCl_3$, $HgCl_2$, HCHO, toluene and $K_2Cr_2O_7$ prevent phosphatase activity while penicillin and terramycin are without effect.

H. J. Velander

PHYSIOLOGY AND ENDOCRINOLOGY

1051. Use of glutamine by the mammary gland for the synthesis of casein. J. M. Barry. Nature, 174, 4424: 315. 1954.

Samples of milk were collected after intravenous injection of C^{14} : N^{15} amide DL-glutamine. Based on the specific activities of the blood plasma and the casein hydrolyzed by acid and by enzyme, it was concluded that the glutamine residues of the casein are derived from the free glutamine of the plasma, and that those derived from glutamic acid come from some other source. R. Whitaker

1052. The rat as a test animal in evaluating artificial human milk for infants. P. Roine, H. Gyllenberg, M. Rosslander, and R. Vuokila. Nature, 174, 4428: 519. 1954.

The pH of the feces of rats was selected as a means of evaluating various modifications of cows' milk to produce the same pH as found in the stools of infants on mothers milk. Results indicated that the desired pH could be obtained in the feces of rats receiving the test milks *ad libitum*. No water was given and no vitamin or mineral adjustments were made in the diet. It was found that the best modification is to increase the lactose content 5-10% and to decrease the Ca content. Ca was reduced by 30% by cation exchange treatment with ZeoKarb 215. Ionizable Ca was reduced by pption. with sodium sulphate, oxalate, or carbonate, and with hexametaphosphate (Calgon). The addition of the last mentioned substance and lactose seems the most suitable means of modifying cows' milk for infants and this subject is undergoing study. R. Whitaker

SANITATION AND CLEANSING

1053. Horsefly and horn fly repellent tests on cattle in New York. W. J. Goodwin, S. Moore III, and H. H. Schwardt, Cornell Univ., Ithaca, N. Y. J. Econ. Entomol., 46, 6: 1088. 1953.

Four materials were tested as emulsion spray repellents against horse flies and horn flies. Cattle were sprayed on July 2, July 17, and August 12 with one quart of repellent. Treatment times coincided with peak numbers of three important species of house flies. Pyrenone (1-10) was the most effective repellent of horse and horn flies. The effective period was 5 to 7 d. Butoxypolypropylene glycol was slightly less effective. E. H. Fisher

1054. Some new fly repellents from laboratory screening tests. L. D. Goodhue and R. E. Stansbury, Phillips Petroleum Co., Bartlesville, Okla. J. Econ. Entomol., 46, 6: 982. 1953.

Four chemicals out of more than 500 laboratory-tested for repellency against the house fly were good. These were diethyl isocinchomeronate, di-n-propyl isocinchomeronate, mercaptopolyoxyethylenes, and butadienefurfural copolymer. These four repellents were laboratory-tested against the stable fly. The second compound above was best—the last two were also good. E. H. Fisher

1055. Baits for the control of resistant flies. J. B. Gahan, R. S. Anders, H. Highland, and H. G. Wilson, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., **46**, 6: 965. 1953.

Laboratory and field tests in dairies evaluated several organic and inorganic compounds in baits against chlorinated hydrocarbon-resistant house flies. In dairies, baits were either sprinkled on the floor or exposed in wire-covered pans which were placed in areas where flies congregate.

TEPP and sodium arsenate each proved more successful than other materials. The addition of either blackstrap molasses or brewers' malt to the toxicant in a water bait proved advantageous. Regular application was necessary to maintain good control. E. H. Fisher

1056. The relative toxicity to house flies of the methyl and ethyl analogs of allethrin. W. A. Gersdorff and N. Mitlin, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., **46**, 6: 945. 1953.

Laboratory tests with house flies compared some toxicants. The methyl analog and ethyl analog of allethrin were 0.12 and 0.26% as toxic respectively as allethrin; they were respectively 0.40 and 0.94 as toxic as pyrethrins. With relation to chemical structure, it was concluded that an unsaturated chain in position 2 of the cyclopentenolone nucleus of pyrethroids is not necessary for high toxicity.

E. H. Fisher

1057. The role of chlorinated polyphenyls in improving lindane residues. I. Hornstein and W. N. Sullivan, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., **46**, 6: 937. 1953.

A mixture of chlorinated terphenyls was used as a film-forming carrier to extend the period of insecticidal efficiency of lindane. The material is a solvent for lindane, lowers lindane vapor pressure, and is inert. Low-boiling solvents such as methylene chloride and even xylene improved the surface coating of lindane-chlorinated terphenyl mixture on a porous surface by minimizing absorption. E. H. Fisher

1058. Microbioassay of insecticides in milk by a feeding method. J-Y. T. Sun and Y-P. Sun, Shell Development Co., Denver, Colo. J. Econ. Entomol., **46**, 6: 927. 1953.

A microbioassay method to determine the amount of an insecticide in milk was developed. Amounts, in p.p.m., detected were as low as 0.1 of aldrin or dieldrin, 0.2 of lindane, 0.5 to 0.6 of isodrin or endrin, and 1.4 of DDT in milk. House flies were used as test insects.

E. H. Fisher

1059. Non-clogging foam-safe aerators lick cheese-waste problem. K. L. Schulze, Intern. Agr. Prod., Inc., Green Bay, Wis. Food Eng., **26**, 9: 51. 1954.

A dairy-waste treatment system featuring specially designed rotors that intimately mix air with whey gives high BOD reduction. Where other aeration systems use from 950-2000 cfm. per lb. raw BOD, the system described uses 200-450 cfm. to achieve the same results. A minimum of 2 aeration tanks is employed. Each is equipped with a specially designed non-clogging rotor which is suspended immediately below the discharge of a down draft tube through which incoming waste is fed. Air is dispersed into the liquid through nozzles in the rotor. Problems from foam and clogging are minimized. Details of operating the system are included. T. J. Claydon

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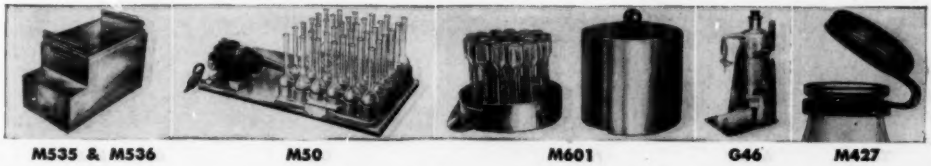

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